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EVALUADORES

El Comité Editorial dentro de sus políticas, envía los artículos a especialistas, con el fin de que sean revisados. Sus observaciones en adición a las que hacen los editores, contribuyen a la obtención de una publicación de reconocida calidad en el ámbito de las Ciencias Agrarias. Sus nombres son mencionados como una expresión de agradecimiento.

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EDITORIAL

Ochenta años de la revista Facultad Nacional de Agronomía, honores y reflexiones

Lord David Attemboroug, tal vez el más destacado científico naturalista de estas últimas cinco décadas, definió al *Homo sapiens* como un "comunicador compulsivo". Y fue acertado: tal vez no haya nada más común a todas las sociedades, países y comunidades, tanto de la remota antigüedad como del mundo moderno, que la comunicación, al punto que es justamente la escritura como lenguaje de intercambio el que define la separación entre la prehistoria y la historia. Comunicar es por excelencia el vehículo de la dimensión cultural que hemos forjado a través de nuestra evolución biológica, que nos permite una relación espacial y nos crea lazos con el pasado y con el futuro.

El editorial del primer número de la Revista Facultad Nacional de Agronomía (RFNA), publicado en agosto de 1939, deja entrever esa necesidad de comunicar los hallazgos y desarrollos en ciencia y tecnología de la Facultad. Decían entonces los profesores Lafaurie y Atehortúa, directores y editorialistas de aquel primer número, que *"… un impetuoso y profundo anhelo de contribuir en algo a la construcción de una Patria vigorosa, fuerte económicamente y por lo tanto libre, nos aúpa para sacar esta Revista …*". Y a fe que así ha sido: ochenta años de publicación ininterrumpida de este que es uno de los patrimonios más acendrados de la centenaria Facultad de Ciencias Agrarias, nos llena de orgullo inmenso, como quiera que es el esfuerzo de muchas generaciones de profesores, de estudiantes y de investigadores ligados a esta unidad académica.

La RFNA ha pasado en estos ochenta años por varios estadios de desarrollo, que se corresponden con los momentos históricos de evolución del sistema universitario colombiano. Antaño eran comunes los artículos de divulgación de experiencias, notas de clase y artículos de opinión, todos con un claro fin de llevar un mensaje directo y práctico a un público muy general que usaba esa información de manera cotidiana; luego fueron tomando forma los artículos de corte técnico, basados en las investigaciones profesorales y estudiantiles; y más recientemente se adoptó el modelo de comunicación cientificista, reglado por normas internacionales, que dirigen su mensaje exclusivamente a la comunidad científica, dejando al margen al técnico o profesional corriente. Esa evolución es lógica, pero pudo haber creado un vacío lamentable: no mantener un vínculo de comunicación con otros usuarios distintos de los científicos y académicos, quienes en su variedad de sitios de desempeño profesional podrían beneficiarse de mucha de la información derivada de los proyectos de investigación y de extensión que se desarrollan en la Facultad.

Celebramos en este año 2019, con sumo orgullo, el haber arribado al octogésimo aniversario de la Revista Facultad Nacional de Agronomía, pero creo que nos debemos una reflexión autocrítica, en el sentido de revisar hacia quiénes y hacia dónde debemos dirigir el acervo y el conocimiento que se deriva de la vida académica de la Facultad. Porque como lo dijera en su momento Lafaurie y Atehortúa, sigue vigente para la Facultad de Ciencias Agrarias *"… un impetuoso y profundo anhelo de contribuir en algo a la construcción de una Patria vigorosa, fuerte económicamente y por lo tanto libre …*".

Guillermo Vásquez Velásquez
Decano
Facultad de Ciencias Agrarias

Research article

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Revista Facultad Nacional de Agronomía

Complete genome sequence of a *Passion fruit yellow mosaic virus* (PFYMV) isolate infecting purple passion fruit (*Passiflora edulis f. edulis*)

UNIVERSIDAD NACIONAL DE COLOMBIA

Secuenciación del genoma completo de un aislamiento del *Passion fruit yellow mosaic virus* (PFYMV) que infecta gulupa (*Passiflora edulis f. edulis*)

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ABSTRACT

Purple passion fruit (*Passiflora edulis* f. *edulis*), also known as gulupa, is a vine plant of the family Passifloraceae, which in recent years has gained importance in the world fruit market due to its exotic nature and excellent organoleptic properties. Although the demand for gulupa in Colombia has increased significantly to become one of the most important fruit exports, the cultivated area has been in decline since 2009 due to the impact of plant diseases. *Cucumber mosaic virus* (CMV), *Soybean mosaic virus* (SMV) and *Cowpea aphid-borne mosaic virus* (CABMV) are amongst the main viruses found infecting gulupa in Colombia. To further characterize the virome of gulupa, a deep sequencing transcriptome study was performed from a producing region in eastern Antioquia. Based on the results of next-generation sequencing (NGS), we report the genome sequence of a tymovirus infecting this plant. Phylogenetic analysis revealed this virus to be a close relative of *Passion fruit yellow mosaic virus* (PFYMV), *Cassia yellow mosaic-associated virus* (CYMaV) and *Calopogonium yellow vein virus* (CYVV). To date, only a 1,115 nt segment comprising the RdRp-CP region of PFYMV has been reported; this sequence shares 84.79% and 95.24% identities at the nucleotide and amino acid levels with isolate PFYMV_Antioquia. Finally, RT-qPCR and Sanger sequencing using specific primers confirmed the presence of PFYMV in different purple passion fruit crops in Antioquia. The present work is the first report of a complete genome sequence of PFYMV.

Keywords:
Next-generation sequencing
Passifloraceae
Tymovirus
Viral genomes

RESUMEN

El maracuyá morado (*Passiflora edulis* f. *edulis*), también conocido como gulupa, es una planta enredadera de la familia Passifloraceae, que recientemente ha tomado importancia en el mercado mundial de frutas exóticas, gracias a sus excelentes características organolépticas. Aunque la demanda de gulupa en Colombia se ha incrementado significativamente en los últimos años al ser una de las frutas que más exporta el país, su área cultivada ha venido reduciéndose desde 2009 debido al impacto negativo de diferentes enfermedades. Hasta el momento se conocía que el *Cucumber mosaic virus* (CMV), *Soybean mosaic virus* (SMV) y *Cowpea aphid-borne mosaic virus* (CABMV) eran los principales virus que afectaban este cultivo en Colombia. Con el fin de continuar caracterizando el viroma de la gulupa, en el presente estudio se realizó una secuenciación del transcriptoma de plantas cultivadas en el oriente de Antioquia. Con base en los resultados obtenidos para la secuenciación de nueva generación (NGS), se detectó un tymovirus infectando estas plantas. Los análisis filogenéticos indicaron que dicho virus está relacionado con: *Passion fruit yellow mosaic virus* (PFYMV), *Cassia yellow mosaic-associated virus* (CYMaV) y *Calopogonium yellow vein virus* (CYVV). La secuencia aquí reportada compartió niveles de identidad de 84,79% y 95,24% en nucleótidos y aminoácidos, con una secuencia de 1115 nt del segmento que comprende la region RdRP-CP del aislamiento PFYMV_Antioquia. Finalmente, mediante reacciones de RT-qPCR y secuenciación Sanger utilizando cebadores específicos, se confirmó la presencia del PFYMV en diferentes cultivos de gulupa de Antioquia. Este es el primer reporte de un genoma completo del PFYMV.

Palabras clave:
Secuenciación de nueva generación
Passifloraceae
Tymovirus
Genomas virales

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Purple passion fruit (*Passiflora edulis* f. *edulis*), also known as gulupa, is a vine plant of the family Passifloraceae, which in recent years has gained importance in the world fruit market due to its exotic nature, excellent organoleptic properties and high content of bioactive compounds (Dhawan *et al*., 2004; Fischer and Rezende, 2008). Purple passion fruit is closely related to yellow passion fruit (*P. edulis* f. *flavicarpa*) but has a sweeter taste and is less acidic due to a higher content of sugars and a lower concentration of citric acid (Morton, 1987; Manicom *et al*., 2003). The gulupa fruit is also a good source of iron, vitamins A, B and C, amino acids, carotenoids and flavonoids and can be eaten fresh or used in the preparation of beverages, jams, chutneys and desserts in general (Morton, 1987; Dhawan *et al*., 2004; Rodríguez-Amaya, 2012). The leaves of purple passion fruit are frequently used in infusions due to their sedative and anti-inflammatory properties (Morton 1987; Ferreres *et al*., 2007, da Silva *et al*., 2013); the flowers, on the other hand, are often used as ornamentals (Manicom *et al*., 2003).

The origin of purple passion fruit has been located to an area comprising southern Brazil, Paraguay and northern Argentina (Morton, 1987) and its cultivation is restricted to tropical and subtropical regions at altitudes between 1400-2500 MASL with temperatures in the 15 to 30 °C range (Jiménez *et al*., 2012). Gulupa is mainly cultivated in Colombia, Ecuador, Peru, South Africa, Kenya, Zimbabwe, India, New Zealand, Sri Lanka, Uganda, Australia and some Pacific islands (Fischer and Rezende, 2008). Total production of passiflora fruits is estimated at 805,000 t year^{-1}, with Brazil (56%), Ecuador (31.0%), and Colombia (3.7%) as the biggest producers (Manicom *et al*., 2003; Fischer and Rezende, 2008; Rodríguez-Amaya, 2012).

During the past decade, the demand of gulupa in Colombia has increased significantly to become one of the most important exotic fruit exports in the country, just behind banana and Cape gooseberry (Jiménez *et al*., 2012). The total cultivated area of gulupa in Colombia comprises approximately 500 ha mostly concentrated in the provinces of Antioquia (36.87%), Cundinamarca (28.8%) and Boyacá (13.51%) and smaller amounts produced in Valle del Cauca, Magdalena, Huila and Santander (Guerrero *et al*., 2011; Agronet, 2015). In spite of the increasing demand, the cultivated area of gulupa has been in decline since 2009 due to the impact of fungal, bacterial and viral diseases. This effect has been most dramatic in the province of Cundinamarca where the total cultivated area went down from 630 ha in 2009 to 204 ha in 2013, and it has had one of the poorest yields in the country (Agronet, 2015). In 2010, a study on plants showing symptoms of viral infection in the Sumapaz region of Cundinamarca confirmed the infection of gulupa by the potyvirus *Soybean mosaic virus* (SMV) (Camelo, 2010). Further work, reported SMV incidences of 23.7 and 27.8% in two gulupa producing regions of Cundinamarca (Gordillo, 2011). Besides SMV, there are reports of gulupa infections by another potyvirus, *Cowpea aphid-borne mosaic virus* (CABMV), which was detected using ELISA in leaves showing mosaic symptoms in Cundinamarca and Valle del Cauca (Gordillo, 2011).

In spite of ranking third in the cultivated area (74 ha), Antioquia has the highest yield of purple passion fruit (31.2 t ha^{-1}) and is also the largest producer with 36.8% of the national production (Agronet, 2015). Unfortunately, commercial gulupa cultivation plots in this region have started to be affected by an unknown disease that results in significant yield reductions. The symptoms of this pathology have been previously observed in gulupa fields in Cundinamarca and are suggestive of an emergent disease of viral origin. A Next-generation sequencing (NGS) study on gulupa fields in the municipality of Jardin demonstrated the presence of a SMV strain infecting this plant in Antioquia and reported the first complete SMV genome infecting a host different to soybean (Jaramillo, 2017). As different viruses have been found to infect *P. edulis* in the world (i.e., *Cucumber mosaic virus*, *Passionfruit mottle virus*, *Bean yellow mosaic virus*), it is likely that other viruses could be found infecting this crop in Antioquia as a result of the free movement of infected plants and the lack of seed certification programs in Colombia. To further characterize the virome infecting gulupa in Antioquia, in this study a NGS analysis was performed from a producing region in the eastern municipalities of El Retiro and La Ceja. The molecular properties of this virus are discussed and its widespread presence in this region demonstrated by RT-PCR and RT-qPCR tests.

MATERIALS AND METHODS

Next-generation Sequencing

High-throughput sequencing of the gulupa transcriptome was performed on a bulked sample of two leaves from approximately 10 plants with symptoms of viral infection such as mosaic, mottling along the veins, enation and severe leaflet deformation (Figure 1) collected at the municipalities of El Retiro (6°03'15.60" N75°30'5.39"W) and La Ceja (6°01'39.4"N, 75°25'53.8"W) (Antioquia, Colombia). After grinding the leaf tissue with liquid nitrogen, total RNA was extracted with Trizol (Ambion, Thermo Fisher Scientific, EEUU) and rRNA depleted with the Ribo-Zero Plant kit (Illumina, EEUU). Libraries were constructed using the TruSeq RNA Sample Preparation Kit (Illumina, EEUU) and sequencing performed with the Illumina HiSeq 2500 system provided by Macrogen (South Korea) (Muñoz-Baena *et al*., 2017). Adapter sequences and low-quality bases were removed with Trimommatic (Bolger *et al*., 2014). Sequence assembly was performed with Trinity (Grabherr *et al*., 2011) and viral contigs identified by a local BLASTN search using a database of viral reference genomes. The database comprised a collection of 10,884 completely sequenced plant viruses representative of all the taxa and genome types currently known for this group of pathogens and were downloaded from NCBI. The PFYMV genome assembly was confirmed by mapping with BWA (Li and Durbin, 2009) using the Trinity contig as the reference. Sequencing of the purple passion fruit transcriptome resulted in a paired-end library of 21,480,984 reads (101 nt/read) for a total of 2,169,579,384 sequenced nucleotides. The consensus genome sequence was deposited in GenBank under accession KY823429 with PFYMV_Antioquia as isolate name.

Figure 1. Leaf symptoms of virus infection in *P. edulis* f. *edulis;* A. Asymptomatic leaf; B. Mottling along the veins; C. Mosaic and enation; D. Severe leaflet deformation.

RT-PCR and RT-qPCR

To confirm the presence of PFYMV_Antioquia in eastern Antioquia, leaf samples were collected in one gulupa plot (~0.4 ha) in the municipality of La Ceja (LCS= La Ceja symptomatic sample; LCA= La Ceja asymptomatic sample) and three more (<1 ha) in El Retiro (R#S=Retiro symptomatic sample; R#A= Retiro asymptomatic sample) (Antioquia, Colombia); two bulked samples consisting of nearly 20 leaves with and without viral symptoms were randomly collected at each plot. RNA was extracted

with Trizol (Ambion, Thermo Fisher Scientific, EEUU) and eluted in 40 µL of DEPC treated water; the purity and concentration were determined by absorbance readings at 260 and 280 nm using a Nanodrop 2000C (Thermo Fisher Scientific, EEUU). Retrotranscription was performed for 30 min at 50 °C in 20 µL containing 200 U of Maxima Reverse Transcriptase (Thermo Fisher Scientific, EEUU), 1X RT Buffer, 0.5 mM dNTP Mix, 100 pmol of the reverse primer, 20 U of RiboLock RNase Inhibitor and 100-500 ng of total RNA (Muñoz-Baena *et al*., 2017). All primers were designed in this study using the PFYMV_Antioquia genome sequence reported in this work. RT-qPCR amplification of the RNA-dependent RNA polymerase (RdRp) region was carried out with primers Tymo_F_RdRp1 (5'-CGG TAC CCT TCC ACA ACT CC-3') and qTymo_R_RdRp1 (5'-AGG GAG CAG AAG AAT TTC G-3'), targeting a 137 bp fragment of the RdRp region; primers Tymo_F_CP (5'-AGT CTC CGA CTC AAT CAG CG-3') and qTymo_R_CP (5'-GCG ACG AGA GAG GTG AGT CG-3') were used for the capsid protein (CP) segment, which resulted in a fragment of 90 bp. For RT-PCR, primer Tymo_R_RdRp1 (5'-CCT GGT GTA AGT GAA CAG GGC-3') was used in combination with Tymo_F_RdRp1 resulting in an amplicon of 576 bp and primer Tymo_R_CP (5'-GAA TGG AGT TCG ATC GTG C-3') was used in combination with Tymo_F_CP, resulting in an amplicon of 405 bp.

For the qPCR, the Maxima SYBR Green/ROX qPCR Master Mix (2X) kit (Thermo Fisher Scientific, EEUU) was used in 25 µL of the reaction containing 12.5 µL mix, 10 µL DEPC water, primers at 0.3 µM each and 50-100 ng cDNA. Samples were considered positive if they exhibited fluorescence values higher than the threshold before the 35th cycle. Primer specificity was verified by High-Resolution Melting (HRM) in the 50 and 99 °C range. For RT-PCR, the amplification mix included 17.8 µL water, 1X enzyme buffer, 1.8 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM primers, 1 U Taq DNA polymerase (Thermo Fisher Scientific, EEUU) and 50-100 ng de cDNA in a final volume of 25 µL. The amplification consisted of an initial 3 min incubation at 95 °C, followed by 35 cycles that included denaturation at 94 °C (30 s), annealing at 52 °C (1 min) and extension at 72 °C (1 min); the amplification ended with an extension cycle at 72 °C for 5 min. Amplicon size was determined by 1.8% agarose gel electrophoresis stained with GelRed 1X (Biotium, EEUU) in a Bio Doc Analyze transilluminator (Biometra, Alemania). Sequences of the RT-qPCR and RT-PCR amplification products were confirmed by the Sanger method using an ABI Prism 3730xl sequencer (PE Applied Biosystems, EEUU) at Macrogen (South Korea). Sanger sequences were deposited in GenBank under accession codes KY823430-KY823445.

Bioinformatic analysis

Open reading frames (ORF) were identified by comparison against known tymoviral sequences using BLASTX with default parameters (scoring matrix: BLOSUM62, word size: 6, gap opening: 11, gap extension: 1) with the exception of E-value, which was set up with a cut-off of 1e-10 (Gish and States, 1993). Functional domains were identified by a search against the Pfam database using the Conserved Domains search at NCBI using default parameters with an expect value threshold of 0.01 (Marchler-Bauer *et al*., 2017). Protease cleavage sites were identified by finding matches to the sequences reported by Jakubiec *et al*. (2007) in *Turnip yellow mosaic virus* (TYMV) using an amino acid sequence alignment with the PFYMV_Antioquia RdRp protein. The 5' hairpin loops 1 and 2 (HP1-2) and the tRNAval-like motif at the 3' end of this tymovirus genome were predicted using the minimum free energy algorithm (Zuker and Stiegler, 1981) available in the ViennaRNA Package (Gruber *et al*., 2008). Evolutionary analyses were conducted in MEGA7 (Kumar *et al*., 2016) using the Maximum Likelihood (ML) method based on the General Reverse Time Reversible model with 1,000 bootstrap replicates (Nei and Kumar, 2000) using a discrete Gamma distribution with five categories. The evolutionary model was selected with ModelTest (Posada and Crandall, 1998). All Sequence alignments were performed with MUSCLE using gap opening and extension penalties of 1 and 0, respectively, a maximum of 16 iterations, and UPGMA as clustering method (Edgar, 2004).

RESULTS AND DISCUSSION

Next-generation Sequencing

Sequencing of the purple passion fruit transcriptome resulted in a total of 2,169,579,384 nucleotides. After sequence assembly, a contig of 6,088 nt with high sequence similarity to members of the genus *Tymovirus* (family *Tymoviridae*) such as PFYMV (84.79%, AF5467107), TYMV (73%, KJ690173), *Grapevine*

fleck virus (GFkV) (73%, JF927942) and *Watercress white vein virus* (WWVV) (71%, JQ001816) (Table 1). Due to high sequence similarity with the partial RdRp (96.3%) and CP (97.0%) protein sequences of PFYMV, the viral contig from *P. edulis* f. *edulis* was named as PFYMV_Antioquia. The PFYMV_Antioquia sequence was assembled from a total of 3,823,738 paired-end reads for an average sequence depth of 123.58x and an abundance of 65,922.13 fragments per kilobase per million reads (FPKM) (Figure 2).

Table 1. Percent nucleotide (nt) and amino acid (a.a.) sequence identities of the PFYMV_Antioquia genome and proteins against selected tymovirus species representing the genus variability. Nucleotide sequence identities are shown for the complete genome and the predicted ORFs encoding the Movement protein (MP), RdRp and CP proteins. Protein comparisons are with respect to predicted amino acid sequences of MP, RdRp, and CP in PFYMV_Antioquia. Dashes represent missing sequences.

	Genome	MP		RdRp		CP	
Tymovirus species	**nt**	**nt**	**a.a.**	**nt**	**a.a.**	**nt**	**a.a.**
Anagyris vein yellowing virus (NC 011559)	56.3	57.0	35.3	57.4	31.8	48.4	37.2
Andean potato latent virus (NC 020470)	55.7	56.5	35.0	55.5	29.1	51.1	39.8
Asclepias asymptomatic virus (NC 015523)	62.5	62.9	41.8	63.0	36.4	58.5	49.2
Chayote mosaic virus (NC 002588)	56.3	57.3	36.5	56.3	31.2	50.6	35.1
Dulcamara mottle virus (NC 007609)	56.2	57.0	35.6	55.0	27.2	49.0	39.8
Eggplant mosaic virus (NC 001480)	57.2	57.9	37.3	55.3	27.5	52.2	41.4
Erysimum latent virus (NC 001977)	54.7	55.5	34.3	56.1	29.5	49.0	34.0
Kennedya yellow mosaic virus (NC 001746)	63.8	62.7	41.9	62.1	36.7	65.9	59.8
Nemesia ring necrosis virus (NC 011538)	55.8	56.7	37.0	55.6	27.8	50.0	37.2
Okra mosaic virus (NC 009532)	63.2	63.1	44.3	60.7	35.2	64.7	59.8
Ononis yellow mosaic virus (NC 001513)	56.2	57.2	37.1	59.4	33.8	45.3	34.5
Physalis mottle virus (NC 003634)	56.7	57.3	36.3	57.2	30.3	49.5	40.9
Plantago mottle virus (NC 011539)	54.8	55.3	34.0	54.4	25.9	48.8	37.2
Scrophularia mottle virus (NC 011537)	55.1	55.8	34.0	55.9	28.9	47.4	37.2
Tomato blistering mosaic virus (NC 021851)	56.3	57.1	38.5	57.6	31.7	47.9	41.5
Turnip yellow mosaic virus (NC 004063)	58.7	58.8	38.7	58.1	33.7	54.8	42.2
Passion fruit yellow mosaic virus (AF467107)*	-	-	-	84.4	96.3	84.9	97.0
Belladonna mottle virus (X54529)	-	-	-	-	-	48.8	34.5
Cacao yellow mosaic virus (X54354)	-	-	-	-	-	64.3	55.3
Calopogonium yellow vein virus (U91413)	-	-	-	-	-	67.1	66.3
Cassia yellow mosaic-associated virus (JN545837)	-	-	-	-	-	67.8	66.4
Clitoria yellow vein virus (AF035200)	-	-	-	-	-	62.7	58.0
Desmodium yellow mottle virus (AF035201)	-	-	-	-	-	61.3	53.4
Petunia vein banding virus (AF210709)	-	-	-	-	-	48.8	37.2
Wild cucumber mosaic virus (AF035633)	-	-	-	-	-	49.7	35.6

*Sequences similarities are based on partial RdRp and CP sequences. For the RdRp, the comparison involves de 3'-end of the ORF comprising residues 5,169-5,418 and 1-250 of PFYMV_Antioquia and entry AF467107. For the CP, the comparison involves 5'-end of the ORF comprising residues 5,422-5,865 and 254-697 of PFYMV_Antioquia and entry AF467107.

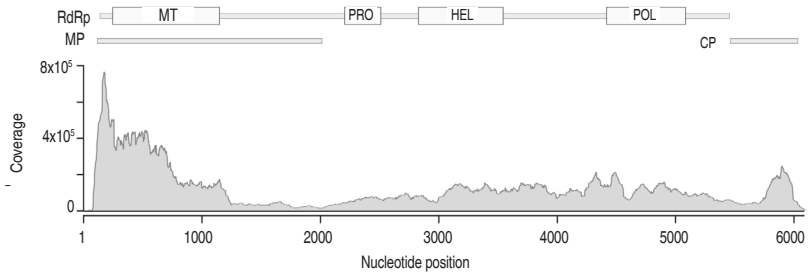

Figure 2. Sequence coverage of the PFYMV_Antioquia genome. The contig was assembled from a total of 3,823,738 paired-end reads for an average sequence depth of 123.58x and an abundance of 65,922.13 fragments per kilobase per million reads (FPKM). The relative position of the ORFs encoding for the MP, RdRp and CP proteins are shown on top and include the location of predicted methyltransferase (MT), protease (PRO), helicase (HEL) and polymerase (POL) domains.

Three ORFs were identified in the PFYMV_Antioquia genome (Figure 3A). ORF1 is located at nucleotide positions 112 to 5,418 and encodes for a putative replication polyprotein of 1,768 a.a. (197.6 kDa) containing methyltransferase (Mtr, pfam01660), helicase (HEL, pfam01443), RNA-dependent RNA polymerase (POL, pfam00978) and protease (PRO, pfam05381) domains. In TYMV, the protease domain

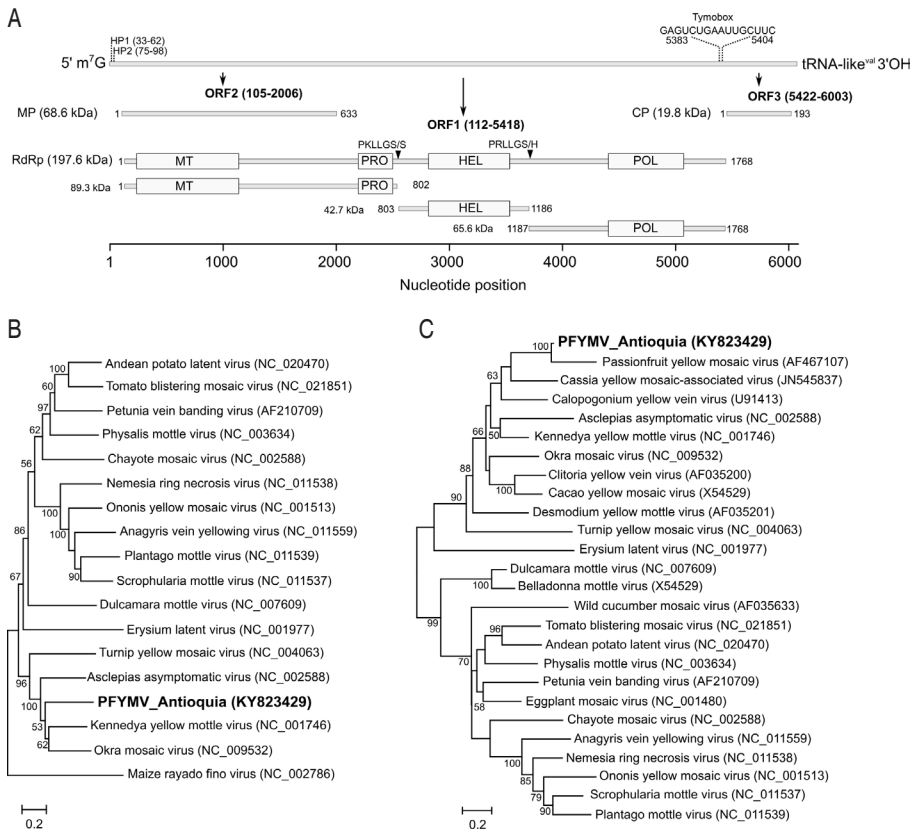

Figure 3. Genome annotation and phylogenetic relationships of the PFYMV isolate from Purple passion fruit in Antioquia. A. Molecular features of the PFYMV_Antioquia genome. The first bar diagram represents the complete genome; nucleotide positions of HP1, HP2 and the tymobox are indicated. The diagrams below are drawn to scale and indicate the position of predicted ORFs and functional domains. Putative protease cleavage sites within the RdRp polyprotein sites are indicated as triangles; B. Maximum-likelihood (ML) phylogenetic tree using available completely sequence tymovirus genomes; C. ML tree using CP sequences. GenBank accession numbers are shown in parentheses.

mediates cleavage of the 206K polyprotein precursor between the HEL/POL and PRO/HEL domains (Jakubiec *et al*., 2007); in the PFYMV_Antioquia genome, the polyprotein is predicted to be cleaved at amino acid positions 802 and 1,186 with recognition sequences PKLLGS/S and PRLLGS/H, respectively. ORF2 is located at positions 105-2,006, 7 nt upstream of ORF1, and encodes a movement protein (MP) of 68.6 kDa. ORF3 was identified at positions 5,422-6,003 and encodes the capsid protein (CP, 19.8 kDa). This protein product is predicted to be translated from a subgenomic mRNA transcribed from a viral promoter, or tymobox, with sequence GAGUCUGAAUUGCUUC and identical to that found in TYMV (Schirawski *et al*., 2000). The tymobox is located at positions 5,383-5,404 within ORF1 and 39 nt upstream of the ORF3 initiation codon (Figure 3A).

In agreement with other tymovirus genomes (Giegé et al., 1993; Bink et al., 2003) RNA secondary structure predictions suggests the formation of two hairpin sequences at positions 33-63 (HP1) and 75-98 (HP2) in the 5' UTR and a tRNA-like structure with a valine anticodon sequence (CAC) in 3' UTR (Figure 4).

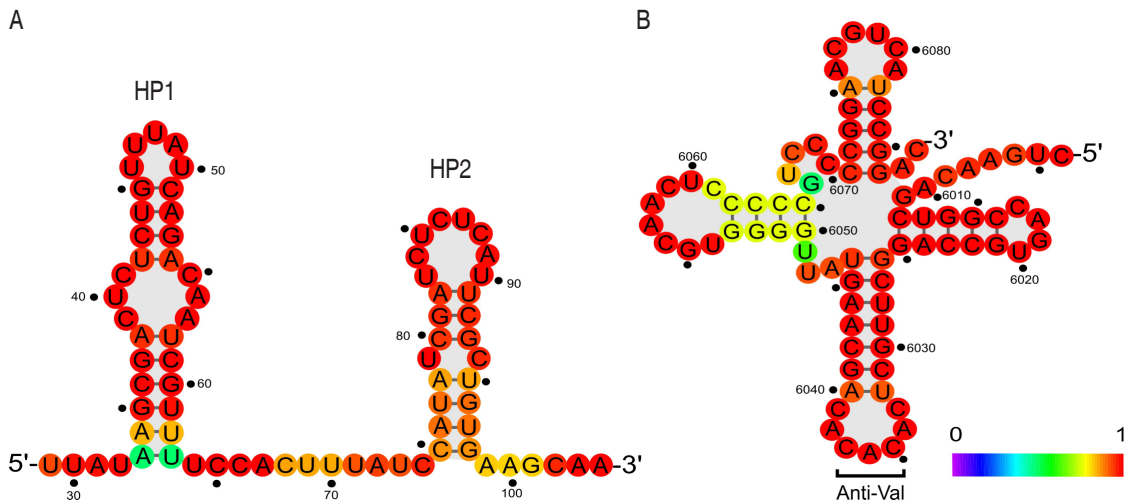

Figure 4. Predicted RNA secondary structure elements in PFYMV_Antioquia. A. Hairpin loops HP1 and HP1. B. $tRNA^{val}$-like structure at the 3'UTR. Secondary structure predictions were performed using the minimum free energy algorithm package available in the ViennaRNA Package. The color scheme represents the probability for a given base to adopt the secondary structure presented in the diagram.

Maximum likelihood analysis of complete tymoviral genomes, using the marafivirus *Maize rayado fino virus* (MRFV) as outgroup, confirms PFYMV_Antioquia to be a member of the genus *Tymovirus* with phylogenetic affinity to a clade comprising *Asclepias asymptomatic virus* (AsAV), *Kennedya yellow mottle virus* (KYMV), *Okra mosaic virus* (OMV) and TYMV (Figure 3B). Analysis of CP sequences confirms that PFYMV_Antioquia is more closely related to PFYMV and forms part of a subclade that also includes *Cassia yellow mosaic-associated virus* (CYMaV) and *Calopogonium yellow vein virus* (CAYVV) (Figure 3C). The only PFYMV sequence available corresponds to a 1,115 nt segment of the 3' end obtained from a passion fruit plant with conspicuous yellow mosaic leaf symptoms in Colombia (Morales *et al*., 2002). It is important to note that the currently available PFYMV sequence (AF5467107) seem to contain errors in the 3'-end of the CP ORF which could mislead future work on PFYMV (Figure 5).

RT-PCR and RT-qPCR

The presence of PFYMV_Antioquia was confirmed in composite samples consisting of either symptomatic (R1S, R2S, R3S, and LCS) or asymptomatic (R1A, R2A, R3A, and LCA) leaves from four purple passion fruit plots in Antioquia using RT-qPCR and RT-PCR with primers targeting RdRp and CP regions (Figure 6). All samples tested positive for this tymovirus. In RT-qPCR, Tymo_F_RdRp1/qTymo_R_RdRp1 reactions exhibited Ct values between 4.16-18.57 and a narrow Tm range (83.6±0.15

°C), while reactions for Tymo_F_CP/qTymo_R_CP had Ct values between 2.33 and 17.72 and Tm of 86.6±0.15 °C, suggesting low sequence variability (Figure 7). Sanger Sequencing of the RT-PCR amplicons and phylogenetic analysis confirmed the presence of the PFYMV (Figure 7).

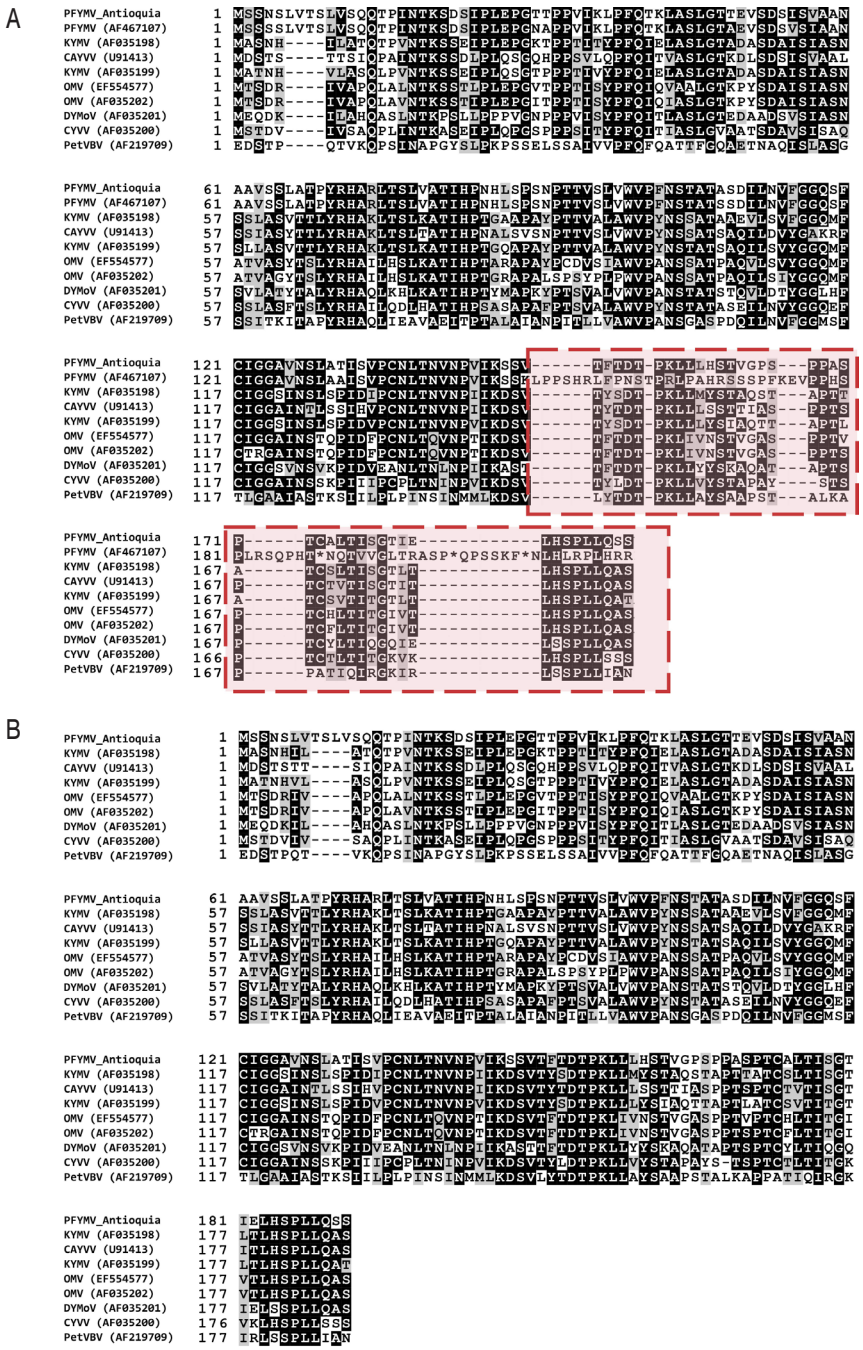

Figure 5. Multiple sequence alignment of the CP region of tymovirus closely related to PFYMV_Antioquia. A. Despite high amino acid sequence identity (97.0%) at the N-terminus (1-143) with PFYMV isolate (AF467107), premature codons are observed at the C-terminus suggesting errors in the deposited sequence (in red); B. Removal of the previously sequenced PFYMV segment (AF467107) reveals that the PFYMV_Antioquia is in good agreement with other previously sequenced tymovirus.

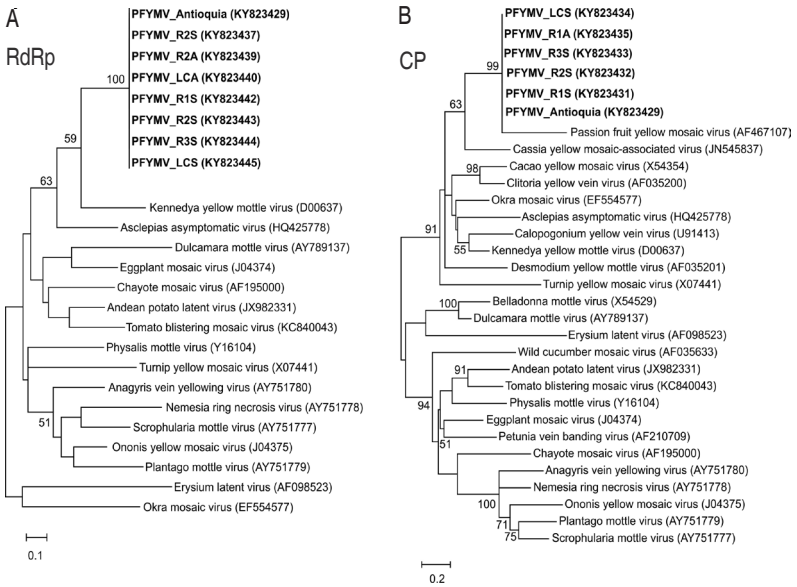

Figure 6. Maximum-likelihood phylogenetic trees of RT-PCR amplicons from PFYMV isolates infecting commercial Purple passion fruit plots. A. ML tree derived from RT-PCR amplification and sequencing of a RdRp segment using primers Tymo_F_RdRp1/Tymo_R_RdRp1. B. ML tree derived from RT-PCR amplification and sequencing of a CP segment using primers Tymo_F_CP/Tymo_R_CP.

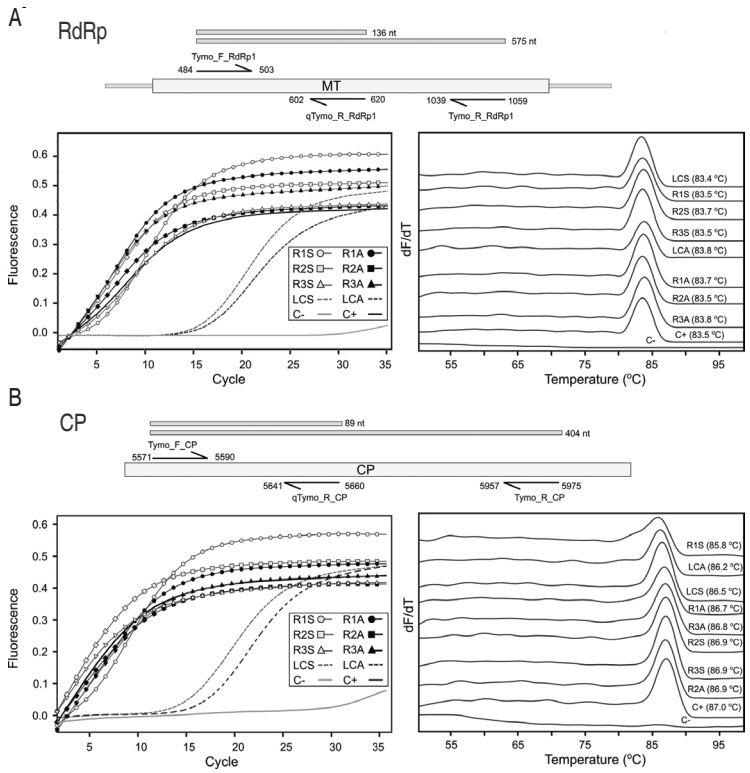

Figure 7. Real-time PCR detection of PFYMV_Antioquia in field samples. Amplifications were performed on symptomatic (R1S, R2S, R3S, and LCS) and asymptomatic (R1A, R2A, R3A, and LCA) bulked leaf samples from commercial *P. edulis* f. *edulis* plots in Antioquia with primers targeting either A. the RdRp or B. CP regions. Primers were designed based on the PFYMV genome presented in this work. Bar diagrams on top of each panel represent the relative position of each primer within the PFYMV genome. Panels to the right and left correspond to amplification curves and melting temperature profiles, respectively

This work confirms the presence of a tymovirus infecting purple passion fruit and phylogenetically related to the PFYMV isolate infecting the yellow passion fruit (*P. edulis* f. *flavicarpa*). Before this work, the only available PFYMV sequence was obtained from passion fruit plants with conspicuous yellow mosaic leaf symptoms in Colombia. Partial genome sequencing showed this isolate to be distinct from other tymoviruses but with similar pathogenic and antigenic properties to PFYMV infecting *Passiflora* spp. (Morales *et al*., 2002). The Brazilian PFYMV isolate was identified in yellow passion fruit plants with symptoms of yellow net, yellow mosaic, and leaf crinkle at Papucaia, in the State of Rio de Janeiro (Crestani *et al*., 1986). The Brazilian PFYMV isolate has a host range limited to species of the genus *Passiflora* and can be transmitted by graft or mechanical inoculation and by the chrysomelid beetle *Diabrotica speciosa* (Crestani *et al*., 1986). The Colombian PFYMV isolate, on the other hand, was first detected in the passion fruit-producing regions of Antioquia, Caldas, Santander and Valle del Cauca as an isometric virus with serological relationship to *Desmodium yellow mottle virus* with particle morphology, which induced symptoms similar to tymoviruses (Varón de Agudelo *et al*., 1992). In contrast to the Brazilian PFYMV, inoculation of the Colombian PFYMV isolate can induce symptoms in other *Passiflora* species such as *Passiflora adenopoda*, *P. edulis*, *P. pinnatistipula*, *P. cuadrangularis* as well as solanaceous plants such as *Physalis angulata*, *P. floridiana* and *P. peruviana* (Morales *et al*., 2002). However, the Colombian PFYMV isolate failed to be transmitted by chrysomelid bettles of the genus *Cerotoma*, *Colaspis* and *Diabrotica* (Morales *et al*., 2002).

This report, on the complete genome sequence of a PFYMV isolate infecting purple passion fruit, is a new addition to the growing number of NGS sequencing reports on viruses affecting *Passiflora* species in South America. For example, a recent work by Vidal *et al*. (2018) showed that blisters, mosaics and leaf deformation symptoms observed in passion fruit crops in Bahia (Brazil) was due to a mixed infection with CABMV and *Cucurbit aphid-borne yellows virus* (CABYV) (*Polerovirus*, *Luteoviridae*). This work is also the first record of CABYV in passion fruit in the world (Vidal *et al*., 2018). Fontenele *et al*. (2018), on the other hand, discovered a divergent geminivirus co-infecting a diseased passion fruit plant together with CABMV in the state of Mato Grosso do Sul (Brazil) using NGS sequencing of circular DNA. This new geminivirus has been tentatively named as Passion fruit chlorotic mottle virus (PCMoV) and is the third ssDNA virus affecting *Passiflora* species together with Passion fruit severe leaf distortion virus (PSLDV), identified infecting this plant in Brazil and Passion fruit leaf distortion virus (PLDV), a proposed species found on *Passiflora edulis* in the province of Valle del Cauca (Colombia) (Vaca-Vaca *et al*., 2017). Finally, Jaramillo *et al*. (2018) reported the complete genome sequence of a SMV strain infecting purple passion fruit in south eastern Antioquia (Colombia) and associated with variegation, rugose mosaics and dark green islands symptoms using RNAseq of symptomatic leaves.

We have given evidence for the existence of a new PFYMV isolate within the genus *Tymovirus*. The species demarcation criteria within this genus are: overall genome sequence identity of less than 80%, capsid protein sequences less than 90% identical, differences in the 3'-terminal structure, differential host range and serological specificity (King *et al*., 2012). At the genome level, the PFYMV_Antioquia shares between 54.8% to 63.8% nucleotide sequence identities with available complete genome sequences of tymovirus being most similar to AsAV (62.5%), KYMV (63.8%) and OkMV (63.2%). A comparison of sequences of the capsid protein reveals that the more closely related species are CYMaV and CYVV with 66.4% and 66.3% amino acid sequence identity, respectively. Tymoviruses have a very restrictive host range and can be transmitted mechanically or by beetles of the families Chrysomelidae and Curculionidae in a semi-persistent manner (King *et al*., 2012).

CONCLUSIONS

Using Next-generation sequencing of the gulupa transcriptome from a bulk sample of leaves from eastern Antioquia, a new isolate of *Passion fruit yellow mosaic virus* (PFYMV) was identified and its complete genome sequence of 6,088 nt was obtained. To our knowledge, this represents the first complete PFYMV genome in the world. The three ORFs of tymoviruses were identified: ORF1 encodes a replication polyprotein of 1,768 a.a. (197.6 kDa), ORF2 encodes the movement protein

(MP), it is located 7 nt upstream of ORF1 and has a predicted molecular weight of 68.6 kDa (633 a.a.) and finally, ORF3 encodes a coat protein (CP) of 19.8 kDa.

Two primer sets targeting the RdRp polyprotein and CP were designed using the PFYMV_Antioquia genome as the reference to confirm the presence of PFYMV by RT-qPCR. Primers were validated using the NGS sample as a positive control and tested in composite samples of both symptomatic and asymptomatic leaves from gulupa plots. Except for the negative control, all samples tested positive for PFYMV. Further work should investigate the host range and vector transmission of PFYMV as well as its serological specificity.

ACKNOWLEDGEMENTS

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REFERENCES

Agronet. 2015. Área, producción y rendimiento de Gulupa en Colombia. In: Agronet, http://www.agronet.gov.co/estadistica/Paginas/default.aspx; accessed: November 2016.

Bink HH, Schirawski J, Haenni AL and Pleij CW. 2003. The 5'-proximal hairpin of turnip yellow mosaic virus RNA: its role in translation and encapsidation. Journal of Virology 77(13): 7452-7458. doi: 10.1128/JVI.77.13.7452-7458. 2003

Bolger AM, Lohse M and Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30(15): 2114-2120. doi: 10.1093/bioinformatics/btu170

Camelo VM. 2010. Detección e identificación de los virus patógenos de cultivos de gulupa (*Passiflora edulis* Sims) en la región del Sumapaz (Cundinamarca). Master's Thesis in Phytopathology. Faculty of Agronomy. Universidad Nacional de Colombia. Bogotá. 60 p.

Crestani OA, Kitajima EW, Lin MT and Marinho VLA. 1986. Passion fruit yellow mosaic virus, a new tymovirus found in Brazil. Phytopathology 76: 951-955. doi: 10.1094/Phyto-76-951

da Silva JK, Cazarin CBB, Colomeu TC, Batista AG, Meletti LMM, Paschoal JAR, Bogusz Júnior S, Furlan MF, Reyes FGR, Augusto F, Maróstica M and Zollnerb R. 2013. Antioxidant activity of aqueous extract of passion fruit (*Passiflora edulis*) leaves: *In vitro* and *in vivo* study. Food Research International 53: 882-890. doi: 10.1016/j.foodres.2012.12.043

Dhawan K, Dhawan S and Sharma A. 2004. *Passiflora*: a review update. Journal of Ethnopharmacology 94(1): 1-23. doi: 10.1016/j.jep.2004.02.023

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792-1797. doi: 10.1093/nar/gkh340

Ferreres F, Sousa C, Valentão P, Andrade PB, Seabra RM and Gil-Izquierdo A. 2007. New C-deoxyhexosyl flavones and antioxidant properties of *Passiflora edulis* leaf extract. Journal of Agriculture and Food Chemistry 55(25): 10187-10193. doi: 10.1021/jf072119y

Fischer IH and Rezende JAM. 2008. Diseases of Passion Flower (*Passiflora* spp.). Pest Technology 2(1): 1-19.

Fontenele RS, Abreu RA, Lamas NS, Alves-Freitas DMT, Vidal AH, Poppiel RR, Melo FL, Lacorte C, Martin DP, Campos MA, Varsani A and Ribeiro SG. 2018. Passion fruit chlorotic mottle virus: molecular characterization of a new divergent geminivirus in Brazil. Viruses 10(4): E169. doi: 10.3390/v10040169.

Giegé R, Florentz C and Dreher TW. 1993. The TYMV tRNA-like structure. Biochimie 75(7): 569-582. doi: 10.1016/0300-9084(93)90063-X

Gish W and States DJ. 1993. Identification of protein coding regions by database similarity search. Nature Genetics 3: 266-272. doi: 10.1038/ng0393-266

Gordillo LA. 2011. Incidencia del Soybean mosaic virus en cultivos de Gulupa (*Passiflora edulis* Sims) en Cundinamarca y estudio de su diversidad en Colombia. Master's Thesis in Microbiology. Faculty of Sciences. Universidad Nacional de Colombia. Bogotá. 106 p.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N and Regev A. 2011. Full-length transcriptome assembly from RNA-seq data without a reference genome. Nature Biotechnology 29: 644-652. doi:10.1038/nbt.1883

Guerrero E, Potosí Guampe C, Melgarejo LM and Hoyos-Carvajal L. 2011. Chapter 7: Manejo agronómico de gulupa (*Passiflora edulis* Sims) en el marco de las Buenas Prácticas Agrícolas (BPA). pp 123-144. In: Melgarejo LM (ed). Ecofisiología del cultivo de la gulupa (*Passiflora Edulis* Sims). Produmedios, Bogotá. 144p.

Gruber AR, Lorenz R, Bernhart SH, Neuböck R and Hofacker IL. 2008. The Vienna RNA Websuite. Nucleic Acids Research 36 Suppl. 2: W70-W74. doi: org/10.1093/nar/gkn188

King AMQ. 2012. Virus Taxonomy. Ninth Report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego. pp. 946-948.

Jakubiec A, Drugeon G, Camborde L and Jupin I. 2007. Proteolytic processing of turnip yellow mosaic virus replication proteins and functional impact on infectivity. Journal of Virology 81(20): 11402-11412. doi: 10.1128/JVId.01428-07

Jaramillo H. 2017. Análisis del transcriptoma y viroma de *Passiflora edulis* f. *edulis* en cultivos de Antioquia utilizando métodos de secuenciación de nueva generación. Master's Thesis in Science. Faculty of Sciences. Universidad Nacional de Colombia. Medellín. 255 p.

Jaramillo H, Marín M and Gutiérrez P. 2018. Molecular characterization of Soybean mosaic virus (SMV) infecting Purple passion fruit (*Passiflora edulis* f. edulis) in Antioquia, Colombia. Archives of Phytopathology and Plant Protection. *In press.*

Jiménez Y, Carranza C and Rodríguez M. 2012. Gulupa (*Passiflora edulis* Sims). pp 579-599. In: Fischer G (ed). Manual para el cultivo de frutales en el trópico. Produmedios, Bogotá.

Kumar S, Stecher G and Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33(7): 1870-1874. doi: 10.1093/molbev/msw054

Li H and Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25(14): 1754-1760. doi: 10.1093/bioinformatics/btp324

Manicom B, Ruggiero C, Ploetz RC and Goes A. 2003. Diseases of passion fruit. pp: 413-441. In: Ploetz RC (Ed). Diseases of tropical fruit crops. CAB international, London.

Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, Chitsaz F, Derbyshire MK, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Geer LY and Bryant SH. 2017. CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. Nucleic Acids Research 45(D1): D200-D203. doi: 10.1093/nar/gkw1129

Morton, J. 1987. Passionfruit. pp. 320-328. In: Morton JF (ed). Fruits of warm climates. Miami.

Morales FJ, Lozano I, Castaño M, Arroyave J, Velasco AC and Varon F. 2002. Partial characterization of a tymovirus infecting passion fruit in Colombia, South America. Journal of Phytopathology 150(4-5): 292-296. doi: 10.1046/j.1439-0434.2002.00740.x

Muñoz-Baena L, Marín-Montoya M and Gutiérrez P. 2017. Genome sequencing of two Bell pepper endornavirus (BPEV) variants infecting *Capsicum annuum* in Colombia. Agronomía Colombiana 35(1): 44-53. doi: 10.15446/agron.colomb.v35n1.60626.

Nei M and Kumar S. 2000. Molecular evolution and phylogenetics. Oxford University Press, Oxford. 333 p.

Posada D and Crandall KA. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14(9): 817-818. doi: 10.1093/bioinformatics/14.9.817

Rodríguez-Amaya DB. 2012. Passionfruit. pp 321-332. In: Siddiq M (ed). Tropical and Subtropical Fruits. Postharvest, Physiology, Processing and Packaging. Wiley-Blackwell, New Jersey. 648p.

Schirawski J, Voyatzakis A, Zaccomer B, Bernardi F and Haenni AL. 2000. Identification and functional analysis of the turnip yellow mosaic tymovirus subgenomic promoter. Journal of Virology 74(23): 11073-11080. doi: 10.1128/JVI.74.23.11073-11080.2000

Vaca-Vaca JC, Carrasco-Lozano EC and López-López K. 2017. Molecular identification of a new begomovirus infecting yellow passion fruit (*Passiflora edulis*) in Colombia. Archives of Virology 162(2): 573-576. doi: 10.1007/s00705-016-3098-y

Varón de Agudelo F, Castaño M, Arroyave JA, Velasco AC, Vuillaume C and Morales FJ. 1992. Complejo viral que afecta plantaciones de maracuyá (*Passiflora edulis* Sims) en el Valle del Cauca. Fruits 47: 321-328.

Vidal AH, Sanches MM, Alves-Freitas DMT, Abreu EFM, Lacorte C, Pinheiro-Lima B, Rosa RCC, Jesus ON, Campos MA, Varsani A and Ribeiro SG. 2018. First World Report of Cucurbit aphid-borne yellows virus infecting Passionfruit. Plant Disease, *In press*. doi: 10.1094/PDIS-04-18-0694-PDN.

Zuker M and Stiegler P. 1981. Optimal computer folding of large RNA sequences using thermodynamics and auxiliary information. Nucleic Acids Research 9(1): 133-148.

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Mixture of glufosinate and atrazine for ryegrass (*Lolium multiflorum* Lam.) control and its effect on seeds' quality

Mezcla de glufosinato y atrazina para el control de raigrás (*Lolium multiflorum* Lam.) y su efecto en la calidad de semillas

doi: 10.15446/rfnam.v72n1.69093

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ABSTRACT

Ryegrass management has been difficult by the occurrence of resistant biotypes to several herbicides with different action mechanisms. Since herbicides mixes and rotations are an important alternative for resistant weed management, the objective of this work was to evaluate the interaction of the dose of the herbicides glufosinate and atrazine on ryegrass control and its seeds' quality exposed to their association. For this study, three experiments were carried out using factorial design in field, laboratory, and greenhouse conditions. Two factors (A and B) were evaluated in each experiment, where factor A and B represented the doses of glufosinate and atrazine, respectively. Ryegrass control was evaluated in field experiment, while germination percentage and Emergence Speed Index (ESI), were obtained in laboratory and greenhouse analyses, respectively. The data were submitted to variance analysis ($P \leq 0.05$) and the significant results were analyzed through response surface graphs. For ryegrass control data, the effect of the interaction was analyzed by the Colby method; glufosinate provides efficient ryegrass control, but its association with atrazine reduces the efficiency, being characterized as an antagonism between molecules. Glufosinate herbicide application, independent of atrazine presence, reduced the ryegrass seeds quality at the post-flowering stage.

Keywords:
Antagonism
Glutamine synthetase inhibitor
Lolium multiflorum
Photosystem II inhibitor

RESUMEN

El manejo de raigrás es difícil debido a la ocurrencia de biotipos resistentes a diversos herbicidas con diferentes mecanismos de acción. Dado que la mezcla de herbicidas y sus rotaciones son una alternativa importante para el manejo de malezas resistentes, el objetivo de este trabajo fue evaluar la interacción de las dosis de los herbicidas glufosinato y atrazina para el control de raigrás y la calidad de sus semillas expuestas a su asociación. Se realizaron tres experimentos usando diseño factorial en condiciones de campo, laboratorio e invernadero. Dos factores (A y B) fueron evaluados en cada experimento, donde el factor A y B representaron las dosis de glufosinato y de atrazina, respectivamente. El control de raigrás fue evaluado en el experimento de campo, mientas que el porcentaje de germinación y el Índice de Velocidad de Emergencia (IVE) se determinaron en laboratorio e invernadero, respectivamente. Los datos fueron sometidos a análisis de la varianza ($P \leq 0,05$) y los resultados significativos fueron analizados a través de gráficos de superficie de respuesta. Para los datos de control, el efecto de las interacciones fue analizado por medio del método Colby; el glufosinato proporcionó un eficiente control de raigrás, pero su asociación con atrazina reduce su eficacia, siendo caracterizando como antagonismo entre moléculas. La aplicación del herbicida glufosinato, independiente de la presencia de atrazina, redujo la calidad de las semillas de raigrás en la fase de post-floración.

Palabras clave:
Antagonismo
Inhibidores de glutamina sintetasa
Lolium multiflorum
Inhibidores del fotosistema II

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Ryegrass (*Lolium multiflorum* L.) is a winter grass, with annual cycle, which has high biomass, production of seeds and tolerance to grazing and soil humidity. Ryegrass is widely used as animal fodder, as well as an autumn-winter cover crop providing a high volume of residue/straw to the no-tillage system (Christoffoleti and López, 2003). This species is easily dispersed by biotic and abiotic factors and is characterized as an important weed in crops like wheat, soybean, maize and orchards in different countries (Brunharo and Hanson, 2018; Fernández *et al*., 2017; Peterson *et al*., 2018; Ruchel *et al*., 2015; Yanniccari *et al*., 2015).

The efficacy of a given weed management program in a cultivated area is the key for weed population dynamics because plants that survive to herbicide treatment can complete their life cycle, disperse seeds, and restock the soil seed bank (Rizzardi *et al*., 2015). In this way, burndown herbicide applications, at an advanced stage of plant growth, have great importance in the weed seed inviability, with impact on the soil seed bank (Bae *et al*., 2017), as well as to guarantee a better establishment of a given crop.

For burndown, it is recommended to use broad-spectrum herbicides that promote high control, for this purpose glyphosate is the most used. However, the repetitive use of this herbicide has generated resistant biotypes of weeds, and it is necessary to adopt herbicides with a different action mechanism. Regarding ryegrass, it is reported that it has developed resistance to eight action mechanism in several countries such as Argentina, Brazil, Chile, Denmark, France, Italy, Japan, New Zealand, Spain, Switzerland, United Kingdom, and United States. It was found a ryegrass biotype in Brazil, which presented resistance to the 5-enolpyruvylhikimate-3-phosphate synthase (EPSPs), acetolactate synthase (ALS) and acetyl coenzyme-A carboxasease (ACCase) inhibitors, showing multiple resistant forms among them (Barroso *et al.,* 2018; Henckes, 2018; Mariani *et al*., 2016; Schneider *et al.,* 2016; Heap, 2018). This situation makes it challenging to manage ryegrass, reducing the effective options for its control.

To reduce the initial interference of weeds, many farmers use herbicide mixtures with non-selective herbicides and residual activity on the soil at pre-sowing applications. For ryegrass management, atrazine is one of the best suitable alternatives. It belongs to the Photosystem II Inhibitors (PSII) action mechanism and is mainly absorbed by plant roots (Silva *et al*., 2013). This herbicide can be mixed with non-selective herbicides such as glufosinate, to be applied before corn sowing, without risk of residual activity on the crop establishment. Glufosinate is a herbicide that inhibits the activity of Glutamine Synthetase (GS), acts on the ammonia incorporation in cells by contact with plant tissue due to its low translocation (Latorre *et al*., 2013; Shaner, 2014).

Although herbicide mixtures constitute an important alternative in weed management, it is necessary to know the type of interaction resulting from the mixture. A herbicide mixture is considered synergistic when its added effect is higher than the response predicted by the effect of the isolated applications of each herbicide; additive effect, when the added effect of the isolated applications is similar to the expected effect; and antagonistic when the effect of the blend is less than the effect of individual applications (Staker and Oliver, 1998). Thus, the knowledge of the interaction effects of herbicides mixture is essential for making recommendations of weed management strategies, especially in the current weed resistance scenario.

On fields where EPSPs, ACCase and ALS inhibitors resistant ryegrass were found, it is recommended to use contact herbicides for burndown applications which association usually has an antagonistic effect by destroying the foliar tissues limiting the absorption and translocation of other herbicides in mixes (Hydrick and Shaw, 1994; Vidal *et al*., 2016). The research hypothesis is that the mixture of glufosinate and atrazine may be an alternative for ryegrass management in pre-sowing, especially in areas where multiple herbicide resistant ryegrass biotypes are present. The contact action of glufosinate and the residual atrazine effect in the soil can provide a more extended control period, with influence on seed production of weeds. The objectives of this work were to evaluate the interaction of glufosinate and atrazine herbicides in the mixture on 1) the control of ryegrass biotypes, and 2) the seed quality of ryegrass biotypes exposed to different doses of the herbicide mixture.

MATERIALS AND METHODS

Three experiments were carried out between October 2013 and April 2014. The first experiment (Experiment I) was in field conditions using a randomized complete block

design with four replications. The second (Experiment II) and the third (Experiment III) experiments were carried out in laboratory and greenhouse, respectively, at Universidade Federal de Pelotas in Capão do Leão/RS (31°48'04.13"S, 52°30'09.22"W). A completely randomized design with three and five replications for experiments II and III were used. Different glufosinate dosage (Factor A: 0, 200, 400 and 600 g a.i. ha^{-1}) and atrazine (Factor B: 0, 1000, 2000 and 3000 g a.i. ha^{-1}.), measured in grams of active ingredient, were arranged in a factorial design. The control was evaluated by the zero doses of atrazine and glufosinate in the factorial design.

The herbicide application in Experiment I was made using a backpack precision sprayer pressurized with CO_2, equipped with a nozzle-type fan 110.015 and calibrated to provide 150 L ha^{-1} of spraying volume. In all treatments, 2% mineral oil adjuvant was added to the herbicide treatment. The experimental units consisted of plots of 6 m^2 (3.0x2.0 m), in an area with a natural occurrence of ryegrass weed which did not have cultivation before. At application time, ryegrass plants were in the post-flowering stage.

The percent of weed control at 8, 16 and 24 Days After Treatment (DAT) on a scale of 0 to 100% was recorded. Zero meant the absence of injuries and 100% the death of all plants. To correct the weed phenological stage, it was considered that the plants of the control plots presented approximately 40, 60 and 75% of senescence in the respective evaluation periods.

At 24 DAT, time that coincided with seed maturation, 20-plant seeds of each plot were sampled for experiments II and III. Samples of the replicates of each treatment were grouped to constitute a seed sample per treatment. These seed samples were used to determine the percent of germination (experiment II) and the Emergence Speed Index (ESI) (experiment III) after 131 days of harvest.

For experiment II, the experimental unit was carried out using transparent plastic boxes (i.e. 'gearbox' type), containing two sheets of blotting paper moistened with distilled water in the proportion of 2.5 times the weight of the dry paper. Fifty seeds per treatment were seeded in each 'gearbox' and located in the BOD-type germination chamber at 20 °C during a 12-hour photoperiod. Germination was evaluated by counting germinated seeds according to the Rules for Seed Analysis (RAS) (Ministério da Agricultura, Pecuária e Abastecimento, 2009). Germination results were showed as a percentage of germinated seed with respect to the initial sowed seeds.

Experiment III was carried out in 6 L (40.0x25.0x7.0 cm) volume plastic trays filled with a 50:50 soil and commercial substrate mixture, which constituted the experimental units. Each band, five rows were sown, spaced at 10 cm, where twenty ryegrass seeds were seeded according to the treatment.

To calculate the ESI, daily emergence of emerged seedlings (aerial emergence greater than 1 cm) was achieved until the stabilization of the emergence occurred 25 Days After Sowing (DAS). The ESI was calculated according to the equation proposed by Maguire (1962) as follows:

$$SG=(G1/N1)+(G2/N2)+(G3/N3)+...+(Gn/Nn)$$

Where:
SG: Emergence Speed Index
G1, G2, G3, ..., Gn: Number of seedlings computed in the first, second, third and last count.
N1, N2, N3, ..., Nn: Number of sowing days in the first, second, third and last count.

Data were analyzed using the two-way ANOVA ($P \leq 0.05$). Significant results were analyzed through the construction of response surface graphs, where the atrazine doses were arranged on the x-axis, glufosinate doses on the y-axis and the response variables on the z-axis. To compare the treatments, the values of the minimum significant differences ($P \leq 0.05$) were calculated.

The effect of interactions between the herbicide doses was analyzed by the Colby method (Colby, 1967). In this method, the effect of the mixtures is evaluated by the equation:

$$E=100-\{[(100-X)\times(100-Y)]/100\}$$

Where:
E: Expected value for the herbicide mixture in each dose combination.
X and Y: Control with each herbicide alone.
When the observed effect (O) resulting from the application

of the X+Y mixture is higher, lower or equal to the expected value (E) the synergism, antagonism, or additivity occur respectively. The expected and observed values were compared by *t*-test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Interactions were observed between the factors tested for all variables analyzed. The control percentage of ryegrass plants at 8 DAT increased as glufosinate doses increased; however, they decreased as the atrazine doses increased (Figure 1A). At 16 and 24 DAT, a similar result was observed on glufosinate doses; however, it was observed an increasing percent of control as atrazine doses increased (Figures 1B and 1C). This response may be due to the slower action of atrazine since this herbicide needs to be absorbed by the roots and translocated via xylem to the active site (Silva *et al*., 2013). On the other hand, with glufosinate (a contact action herbicide) shows phytotoxicity in few days after the application.

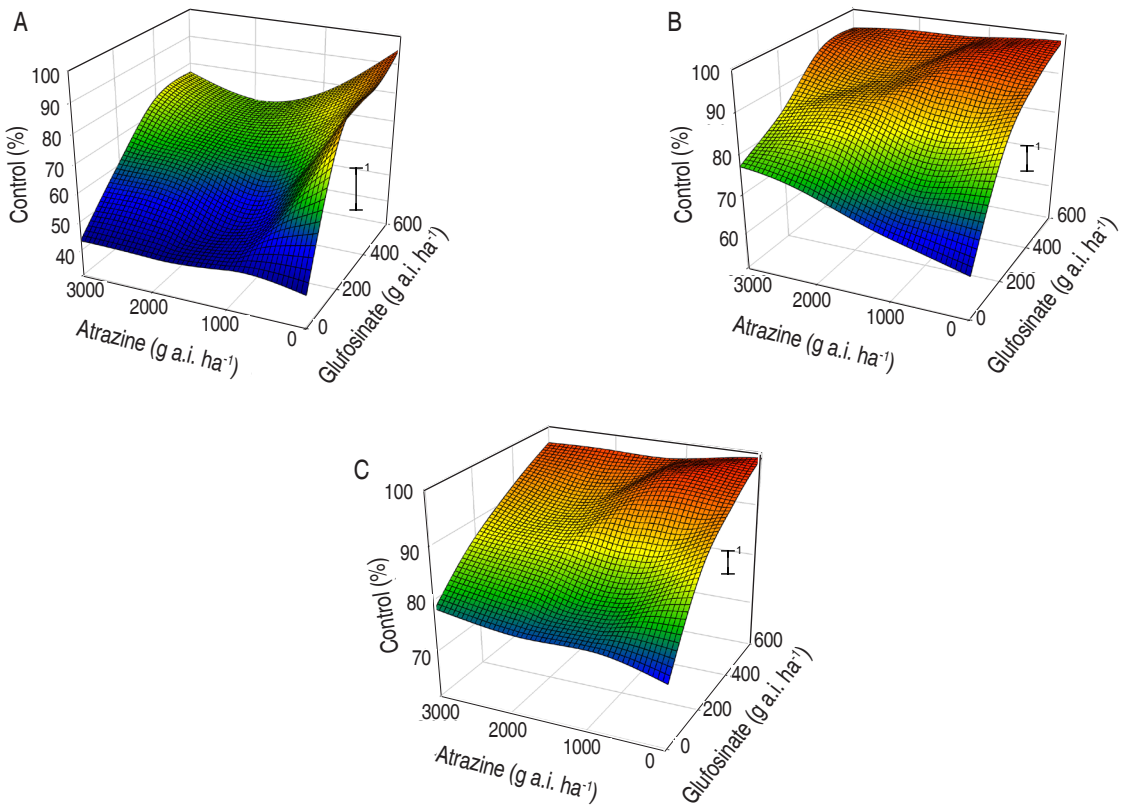

Figure 1. Control percent of ryegrass (*Lolium multiflorum* L.) at A. 8; B.16; C. 24 days after application (DAT) of mixture of glufosinate and atrazine herbicides. Different colors represent a significant difference ($P \leq 0.05$).

Jones *et al*. (2001) showed an increase of 14% in the control of *Amaranthus palmeri* S. Watson with a glufosinate+atrazine mixture in comparison to individual glufosinate. Similarly, Stephenson *et al*. (2015) demonstrated an increase of around 10% of the control of *Abutilon theophrasti* Medik, *Amaranthus palmeri*, and *Ipomoea hederacea* var. *integriuscula* Gray with a mixture of atrazine+glufosinate. However, these researchers also reported no differences between the application of atrazine alone and in mixture with glufosinate in the tank (Stephenson *et al*., 2015). On the other hand, results on species belonging to Poaceae family are different, where glufosinate+atrazine mixture did not adequately control *Sorghum halepense* (L.) Pers and *Urochloa ramosa* (L.) Nguyen, promoting control up to 89%, and these results did not differ from the control by using glufosinate alone (Stephenson *et al*., 2015), evidencing

the low atrazine effect for the control of these species. Therefore, the effect of the interaction between atrazine and glufosinate may be synergistic or antagonistic, depending on the application conditions and weed species.

Concerning the interaction between the two molecules, a decrease in glufosinate efficiency was observed due to the increase atrazine dose, most of the comparisons evidenced antagonism independent of the doses tested (Table 1). Alike results were found for the association of glufosinate and metribuzin where antagonism was observed at low doses of the former (Hydrick and Shaw, 1994), this can be because atrazine and metribuzin have the same action mode.

Table 1. Observed (Obs) and Expected (Exp) control (%) of *Lolium multiflorum* at diferents glufosinate and atrazine doses and the interaction (Int) between herbicides at 8, 16 and 24 Days After Treatment (DAT).

	Glufosinate (g a.i·ha^{-1})											
Atrazine	**0**		**200**			**400**			**600**		**Average**	**Average**
(g a.i.·ha^{-1})	**Obs[1]**	**Obs**	**Exp[1]**	**Int[1]**	**Obs**	**Exp**	**Int**	**Obs**	**Exp**	**Int**	**Obs**	**Exp**
8 DAT												
0	43	86	-		89	-		95	-		78	-
1000	45	53	92*	Ant[2]	69	93*	Ant	77	97*	Ant	61	94
2000	43	59	92*	Ant	67	93*	Ant	70	97*	Ant	60	94
3000	44	61	92*	Ant	75	93*	Ant	77	97*	Ant	64	94
Average	43	65	92		75	93		80	97		66	94
16 DAT												
0	62	85	-		94	-		98	-		85	-
1000	66	84	95*	Ant	95	98*	Ant	97	99*	Ant	85	97
2000	70	87	95*	Ant	90	98*	Ant	96	99^{ns}	Adit	85	98
3000	77	81	97*	Ant	94	99*	Ant	95	100*	Ant	86	98
Average	68	84	96		93	98		96	99		85	98
24 DAT												
0	73	89	-		95	-		99	-		89	-
1000	76	83	97*	Ant	96	99*	Ant	97	100*	Ant	88	99
2000	76	85	97*	Ant	90	99*	Ant	97	100*	Ant	87	99
3000	78	84	98*	Ant	92	99*	Ant	97	100*	Ant	88	99
Average	76	85	97		93	99		97	100		88	99

[1] Obs, exp and nt mean the values observed in the experiment, the values expected by the Colby method and the result of the interaction based on the expected values, respectively. [2]Ant. represents the result of the interaction between the antagonistic herbicides. Adit. indicates the additive interaction. * and ^{ns} indicate significant difference or not between the values observed and expected by the t test ($P \leq 0.05$).

When a mixture of glufosinate and glyphosate was used, a reduction in the control efficacy of *A. theophrasti*, *Chenopodium album* L., *Eleusine indica* Gaert. and *Setaria faberi* Herrm was observed compared to the separate application of glyphosate (Bethke *et al*., 2013; Chuah *et al*., 2008). The predominant antagonistic interactions in the association of these herbicides may occur because molecules with contact action rapidly destroy the leaf tissue, impairing the absorption and translocation of systemic herbicides (Vidal *et al*., 2016).

The application of glufosinate, regardless of atrazine doses in the mixture, when the ryegrass plants were in the post-flowering stage, caused a reduction in the quality of the ryegrass seed, evidenced by germination and ESI decreasing (Figure 2). A similar result was observed with the application

of 600 g a.i. ha^{-1} of glufosinate, which provided an absence of germination in ryegrass seeds (de Campos *et al*., 2012), characterizing as a promising alternative for the management of the species and the reduction of seeds in the soil. For the management of *Eriochloa villosa* Thunb, it was verified a reduction in the viability of seeds when glyphosate was applied, which efficiency only occurred when the application occurred shortly before the inflorescence emission (Nurse *et al*., 2015), demonstrating the importance of the stage at the time of application.

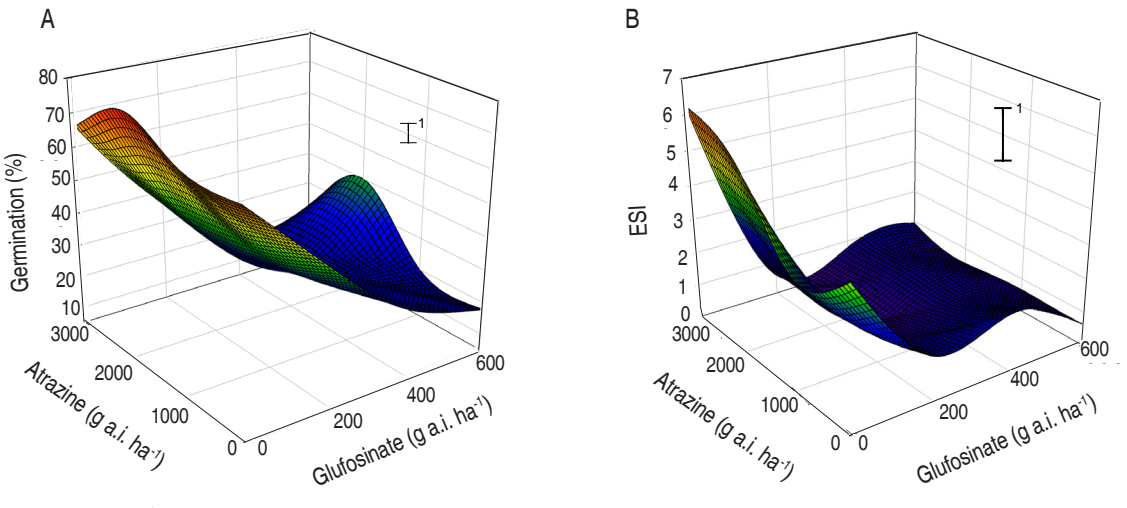

Figure 2. A. Percentage of germination; B. Emergence Speed Index (ESI) of seeds from ryegrass (*Lolium multiflorum* L.) harvested from plants exposed to doses of glufosinate and atrazine herbicides. Different colors represent a significant difference ($P \leq 0.05$).

For *Ambrosia artemisiifolia* L. the stage of development was determinant for the application of For *Ambrosia artemisiifolia* L. the stage of development was determinant for the application of glyphosate and glufosinate to reduce the production of viable seeds and pollen (Gauvrit and Chauvel, 2010). Thus, the burndown with the use of glufosinate at the appropriate time can make the weed seeds unviable, reducing the deposit of viable propagules in the soil seed bank. Although, without interference in the viability of ryegrass seeds, the association with atrazine may be detrimental to the management of the species by reducing control efficiency.

CONCLUSIONS

Glufosinate provides an efficient control of ryegrass at doses of 400 and 600 g a.i.·ha^{-1}; however, the tank mix with atrazine is generally antagonistic and decrease the control efficacy. Ryegrass plants exposed to glufosinate, loss seed viability, reducing germination and Emergence Speed Index (ESI) independently of atrazine.

REFERENCES

Bae J, Nurse RE, Simard MJ and Page ER. 2017. Managing glyphosate-resistant common ragweed (*Ambrosia artemisiifolia* L.): effect of glyphosate-phenoxy tank-mixes on growth, fecundity and seed viability. Weed Science 65(1): 31-40. doi: 10.1614/WS-D-16-00094.1

Barroso AAM, Costa MGS, Neto NJ, Santos JI, Balbuena TS, Carbonari CA and Alves PLCA. 2018. Protein identification before and after glyphosate exposure in *Lolium multiflorum* genotypes. Pest Management Science 74(5): 1125-1133. doi: 10.1002/ps.4831

Bethke RK, Molin WT, Sprague C and Penner D. 2013. Evaluation of the interaction between glyphosate and glufosinate. Weed Science 61(1): 41-47. doi: 10.1614/WS-D-12-00031.1

Brunharo CACG and Hanson BD. 2018. Multiple herbicide-resistant Italian Ryegrass [*Lolium perenne* L. spp. *Multiflorum* (Lam.) Husnot] in California perennial crops: Characterization, mechanism of resistance, and chemical management. Weed Science *In press*. doi: 10.1017/wsc.2018.50

Christoffoleti PJ and López R. 2003. Principais aspectos da resistência de plantas daninhas ao herbicida glyphosate. Planta Daninha 21(3): 507-515. doi: 10.1590/S0100-83582003000300020

Chuah TS, Teh HH, Cha TS and Ismail BS. 2008. Antagonism of glufosinate ammonium activity caused by glyphosate in the tank mixtures used for control of goosegrass (*Eleusine indica* Gaertn.). Plant Protection Quarterly 23(3): 116-119.

Colby SR. 1967. Calculating synergistic and antagonistic responses of herbicide combinations. Weeds 15(1): 20-22. doi: 10.2307/4041058

de Campos CF, Martins D, Costa, ACPR Da, Pereira MRR, Cardoso LA and Martins CC. 2012. Efeito de herbicidas na dessecação e germinação de sementes remanescentes de *Lolium multiflorum* L. Semina: Ciências Agrárias 33(6): 2067-2074. doi: 10.5433/1679-0359.2012v33n6p2067

Fernández P, Alcántara R, Osuna MD, Vila MM and De Prado R. 2017. Forward selection for multiple resistance across the non-selective glyphosate, glufosinate and oxyfluorfen herbicides in *Lolium* weed species. Pest Management Science 73(5): 936-944. doi: 10.1002/ps.4368

Gauvrit C. and Chauvel B. 2010. Sensitivity of *Ambrosia artemisiifolia* to glufosinate and glyphosate at various developmental stages. Weed Research 50(5): 503-510. doi: 10.1111/j.1365-3180.2010.00800.x

Heap I. 2018. Chronological Increase in resistant weeds globally. In: International Survey of Herbicide Resistant Weeds, http://www.weedscience.org; Accessed: October 2018.

Henckes JR. 2018. Resistência múltipla e habilidade competitive de *Lolium multiflorum* Lam. aos herbicidas glifosato (EPSPs), iodosulfurom-metílico e piroxsulam (ALS). Dissertation. Faculdade de Agronomia Eliseu Maciel. Universidade Federal de Pelotas. Capão do Leão. 91 p.

Hydrick DE and Shaw DR. 1994. Effects of tank-mix combinations of non-selective foliar and selective soil-applied herbicides on three weed species. Weed Technology 8(1): 129-133. doi: 10.1017/S0890037X00039324

Jones CA, Chandler JM, Morrison JE, Senseman SA and Tingle CH. 2001. Glufosinate combinations and row spacing for weed control in glufosinate-resistant corn (*Zea mays*). Weed Technology 15(1): 141-147. doi: 10.1614/0890-037X(2001)015[0141:GCARSF]2.0.CO;2

Latorre D, Silva IP, Junior JF, Putti FF, Schimidt AP and Ludwig R. 2013. Herbicidas inibidores da glutamina sintetase. Revista Brasileira de Engenharia de Biossistemas 7(3): 134-141. doi: 10.18011/bioeng2013v7n3p134-141

Maguire JD. 1962. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Science 2(1): 176-177. doi: 10.2135/cropsci1962.0011183X000200020033x

Mariani F, Vargas L, Agostinetto D, Fraga DS, Santos FM and Piesanti SR. 2016. Resistência de biótipos de azevém ao herbicida iodosulfurom-metílico sódio e herbicidas alternativos para o controle. Revista de la Facultad de Agronomía 115(1): 35-43.

Ministério da Agricultura, Pecuária e Abastecimento. 2009. Regras para Análise de Sementes, In: Ministério da Agricultura, Pecuária e Abastecimento, http://www.agricultura.gov.br/assuntos/laboratorios/arquivos-publicacoes-laboratorio/regras-para-analise-de-sementes.pdf/view. 398 p.; Accesed: September 2017.

Nurse RE, Darbyshir SJ and Simard MJ. 2015. Impact of post-anthesis glyphosate on woolly cupgrass seed production, seed weight and seed viability. Canadian Journal of Plant Science 95(6): 1193-1197. doi: 10.4141/cjps-2015-166

Peterson MA, Collavo A, Ovejero R, Shivrain V and Walsh MJ. 2018. The challenge of herbicide resistance around the world: a current summary. Pest Management Science 74(10): 2246-2259. doi: 10.1002/ps.4821

Rizzardi MA, Galvan J, Martins FB and Schneider T. 2015. Ryegrass seed viability in function of phenological stage of herbicides application. Revista Brasileira de Herbicidas 14(3): 111-116. doi: 10.7824/rbh.v14i2.405

Ruchel Q, Vargas L, Agostinetto D, Fernando JA, Lüdtke R and Bobrowski VL. 2015 Caracterização morfoanatômica, contagem cromossômica e viabilidade polínica de biótipos de azevém suscetível e resistentes ao herbicida glyphosate. Planta Daninha 33(3): 567-578. doi: 10.1590/S0100-83582015000300019

Schneider T, Vargas L, Agostinetto D, Langaro AC, da Silva JD and Piessanti SR. 2016. Resistance characterization of Italian ryegrass (*Lolium multiflorum*) biotypes to clethodim herbicide. African Journal of Agricultural Research 11(18): 1593-1600. doi: 10.5897/AJAR2015.10092

Shaner DL. 2014. Herbicide Handbook. 10th edition. Weed Science Society of America, Champaign. 513 p.

Silva IP, Junior JF, Putti FF, Latorre D, Schimidt AP and Ludwig R. 2013. Herbicidas inibidores do fotossistema II – Parte I. Revista Brasileira de Engenharia de Biossistemas 7(1): 1-11. doi: 10.18011/bioeng2013v7n1p12-22

Staker RJ and Oliver LR. 1998. Interaction of glyphosate with chlorimuron, fomesafen, imazethapyr and sulfentrazone. Weed Science 46(6): 652-660.

Stephenson DO, Bond JA, Landry RL and Edwards HM. 2015. Weed management in Corn with Postemergence Applications of Tembotrione or Thiencarbazone: Tembotrione. Weed Technology 29(3): 350-358. doi: 10.1614/WT-D-14-00104.1

Vidal RA, Queiroz ARS, Trezzi MM and Kruse ND. 2016. Association of glyphosate with other agrochemicals: the knowledge synthesis. Revista Brasileira de Herbicidas 15(1): 39-47. doi: 10.7824/rbh.v15i1.428

Yanniccari M, Istilart C, Giménez DO and Castro AM. 2015. Inheritance of glyphosate resistance in *Lolium perenne* and hybrids with *Lolium multiflorum*. Crop Protection 71(1): 72-78. doi: 10.1016/j.cropro.2015.01.024

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Effect of nutrient omission in the development of sunflower BRS-122 in greenhouse conditions

UNIVERSIDAD NACIONAL DE COLOMBIA

Efecto de la omisión de nutrientes en el desarrollo de girasol cultivar BRS-122 en condiciones de invernadero

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is responsible for 13% of all vegetable oil produced in the world. These plants' development depends on the mineral elements that have essential and specific functions in their metabolism. In this sense, visual diagnosis consists of comparing the appearance of a plant that has received all the necessary nutrients with one that has suffered the omission of one or more nutrients. Therefore, this study aimed to evaluate the absence effects of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S) and iron (Fe) elements on the growth of sunflower, BRS-122 cultivar, in order to identify and describe the visual symptoms caused by the absence of such nutrients. The experiment was carried out in a greenhouse and consisted in a completely randomized design with three replications and eight treatments using a diagnostic subtraction technique. The symptoms of the deficiencies were observed and evaluated through biometric parameters (plant height, stem diameter, number of leaves, and leaf area) as well as by visual aspects. The absence of N, P, K, Ca and Fe in the nutrient solution severely affected the sunflower plants, preventing their vegetative growth and consequently their development. The negative interference of the Mg omission in sunflower growth was slower than the observed for nitrogen, phosphorus, potassium, and calcium giving more significant results from 40 days after sowing (DAS). The absence of nutrients gave clear evidence of the distinct effects that the omission of each element can cause on the visual aspects of sunflower plants.

Keywords:
Helianthus annuus
Missing mineral nutrients
Nutrient solution
Visual diagnosis

RESUMEN

El girasol (*Helianthus annuus* L.) es responsable del 13% de todo el aceite vegetal producido en el mundo. El desarrollo de estas plantas depende de los elementos minerales que tienen funciones esenciales y específicas en su metabolismo. En este sentido, el diagnóstico visual consiste en comparar la apariencia de una planta que recibió todos los nutrientes necesarios con una que sufrió la omisión de uno o más nutrientes. Por lo tanto, este estudio tuvo como objetivo evaluar los efectos de la ausencia de nitrógeno (N), fósforo (P), potasio (K), calcio (Ca), magnesio (Mg), azufre (S) y hierro (Fe) en el crecimiento de girasol, cultivar BRS-122, a fin de identificar y describir los síntomas visuales de la ausencia de tales nutrientes. El experimento fue conducido en invernadero y consistió en un diseño completamente al azar, con tres repeticiones y ocho tratamientos, utilizando una técnica de diagnóstico de sustracción. Los síntomas de las deficiencias fueron observados y evaluados a través de parámetros biométricos (altura de la planta, diámetro del tallo, número de hojas y área foliar), así como por aspectos visuales. La ausencia de N, P, K, Ca y Fe en la solución nutritiva afectaron severamente a las plantas de girasol, impidiendo su crecimiento vegetativo y consecuentemente su desarrollo. La interferencia negativa de la omisión del Mg en el crecimiento del girasol fue más lenta que la observada en N, P, K y Ca, con resultados más significativos a partir de los 40 días después de la siembra (DAS). La ausencia de los nutrientes dio claras evidencias de los distintos efectos que la omisión de cada elemento puede causar en los aspectos visuales de las plantas de girasol.

Palabras clave:
Helianthus annuus
Nutrientes minerales faltantes
Solución nutritiva
Diagnóstico visual

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Sunflower (*Helianthus annuus* L.) is an annual plant, originally from the American continent; however, it is grown all over the world. The expansion of its cultivation in all Brazil regions is due to its good adaptation to diverse edaphoclimatic conditions, being characterized by the tolerance to low temperatures in the initial phase of development and by the relative resistance to water deficits. According to Zobiole (2010), the sunflower yield is influenced by latitudes and altitudes as well as by the photoperiod.

The sunflower is responsible for 13% of all vegetable oil produced in the world, which it is currently the fourth most consumed oil in the world after soybean, palm, and canola. Seeds are rich in oil, some sunflower varieties produced by hybridization have amounts higher than 50%, they rarely contain less than 30% (Lira *et al*., 2011). This oil has excellent industrial and nutritional quality, being its primary use as edible oil (Castro *et al*., 1997). Besides, it is an extremely versatile plant, it can be used for animal feed, in human food, and as an ornamental plant (Rodrigues *et al*., 2010).

Mineral elements, such as macronutrients, have essential and specific functions in plant metabolism. Thus, when one of these elements is not present in adequate amounts, or under conditions that make it unavailable, its deficiency in cells promotes changes in plant metabolism manifested by characteristic deficiency symptoms (Taiz and Zaiger, 2009). Therefore, it is recommended to study the effects caused by the lack of mineral elements on sunflower culture, since it has economic relevancy; besides, adequate mineral nutrition of plants is crucial for ideal growth of plants.

For example, Nitrogen (N) is part of the amino acids, proteins, nucleic acids, enzymes, and pigments structure, and it participates in processes of photosynthesis, respiration, multiplication and cellular differentiation (Malavolta *et al*., 1997; Marschner, 1995). Phosphorus (P) participates in the structural formation of plants, in the energy supply to produce photoassimilates and in the quality of final products (Brandão, 2009). Potassium (K) acts directly and indirectly in photosynthesis and respiration, as well as in the food plant transportation. Among nutrients, K is described as having a significant influence in combating plant diseases, as it increases resistance to some pathogens development, it also increases cell wall thickness, provides greater tissue stiffness and promotes rapid recovery after injury (Basseto *et al*., 2007). Calcium (Ca) influences elongation and differentiation of cells (Bergmann, 1992). Ca deficiency can cause meristem death (Marschner, 1995). From the existing nutrients, magnesium (Mg) is essential in photosynthesis because it participates in metabolic processes such as ATP formation in chloroplasts. Magnesium also acts in protein synthesis, chlorophyll formation, phloem loading, photoassimilates separation and use (Marschner, 1995). Sulfur (S) is a secondary anionic macronutrient necessary for the of plants development. S functions are hormonal control for cell growth and differentiation, it supports the plant defense against pests and diseases, and it is an important component for proteins. Iron (Fe) is a micronutrient that acts as an enzyme activator or component, influences the fixation of nitrogen, catalyzes the biosynthesis of chlorophyll, and acts on stems and roots development (Malavolta *et al*., 1997; Marschner, 1995).

Visual diagnosis is used to understand the element absence on plant development. It consists of evaluating and comparing the appearance of a plant that received a solution, with all the necessary nutrients, to another plant that received a solution missing one or more nutrients. In most cases, the leaf appearance is generally analyzed, but it can be analyzed elsewhere in the plant depending on the element absented (Carvalho *et al*., 2001; Malavolta *et al*., 1997). However, before the visible manifestation of the nutrient deficiency, growth and/or production can already be affected by this deficiency, this is what is called hidden hunger, which can only be detected through chemical analysis of the plant material or foliar diagnosis (Malavolta, 2006).

On these bases, the objective of this research was to evaluate the effects of macronutrients and micronutrient (iron) absence on the growth of sunflower, BRS-122 cultivar, and to identify and describe the visual symptoms caused by such nutrients absence.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions at the Department of Agricultural Engineering of Universidade Federal de Campina Grande, from June

to August of 2016, with sunflower plants of the BRS-122 cultivar.

The statistical experimental design was completely randomized, consisting of eight treatments and three replicates, totaling 24 experimental units. Each experimental unit presented a sunflower plant, according to the following treatments: T1- control treatment with the complete solution (CS) according to Hoagland and Arnon (1950), T2- nutritive solution with omission of nitrogen (N), T3- nutritive solution with omission of phosphorus (P), T4-nutritive solution with omission of potassium (K), T5- nutritive solution with omission of calcium (Ca), T6- nutritive solution with omission of magnesium (Mg), T7-nutritive solution with omission of sulfur (S), and T8- nutritive solution with omission of iron (Fe). The only micronutrient evaluated was Fe because, in a previous pilot test designed to define the treatments of this research, it was observed that the omission of the other micronutrients (B, Cu, Mn, Mo, and Zn) did not show symptoms of deficiency in sunflower plants.

The stock nutrient solutions (control) were prepared with guaranteed reagents and deionized water. During the whole experiment, pH and electrical conductivity (EC) measurements were taken to control them, always maintaining pH values in the range of 6.0 to 7.0 and EC around 2.5 dS m^{-1}.

The sunflower plants BRS-122 cultivar used in the experiment were obtained via seeds germinated in phenolic sponges conditioned in a plastic container (disposable cups) with a capacity of 50 mL containing deionized water until the surface of the sponge. Six days after germination, when the formation of four leaves in the seedlings was observed, they were transferred to one-liter pots with the complete nutrient solution established by Hoagland and Arnon (1950), but only with 10% of the ionic strength for the seedlings' adaptation and under constant aeration. In the sequence, the ionic strength of the solution increased to 40% in the second week, increasing gradually up to 100%. After this adaptation period (three weeks), plants were transplanted to one-liter pots, and the treatments were applied under the missing element technique. The pots were capped with expanded polystyrene with a hole in the center of them for the fixation of the crops and another hole in the end for the air entrance.

The evaluations of biometric characteristics such as plant height, stem diameter, and the number of leaves were done at 20, 30, 40 and 50 days after sowing (DAS). The leaf area was evaluated only in the last three samplings. At 50 DAS, plants were harvested and separated in roots and shoot (leaves, branches, and stems) packed in paper bags properly labeled and taken for drying in a forced circulation oven at 65 °C until reaching constant weight, obtaining the dry phytomass weight of roots and shoot.

Visual deficiency symptoms were initially recorded at 20 DAS by photographs and daily described throughout the experimental period (50 DAS) in order to observe the beginning of each deficiency symptom. The data were submitted to analysis of variance and comparison of means, using the Tukey test at 1% of probability level, applying the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

Biometric Characteristics Assessments

The nutritional omissions that affected the most the plant height variable, restricting it, were: Ca, Fe (75.77, 65.43%, respectively) at 30 DAS; K, N, P, Mg, and S (77.05, 71.01, 61.84, 27.54%, and 18.84%, respectively) at 50 DAS, when compared to the complete treatment (control). Due to Ca omission in the nutrient solution, the plants were developed only up to the 30 days of omission, presenting the shortest height comparing to the control treatment, dying after this period (Figure 1). These effects were similar to the iron absence in the nutrient solution of plants.

The plants submitted to treatments with the omission of N, P, and K, maintained a continuous growth throughout the experiment, presented plant height inferior to the control treatment, with heights 3.45, 2.62, and 4.36 times lower than the observed for the control treatment, respectively. The solutions with the omission of Mg and S presented the smallest significant difference concerning the control treatment (Figure 1).

Several authors have observed that the growth of different plant species is affected in the same way when they are growing in solutions lacking nutrients (Prado *et al*., 2007; Maia *et al*., 2011) since the nutrients are fundamental for all the metabolic processes and structural formation in plants, as described in Marschner (1995).

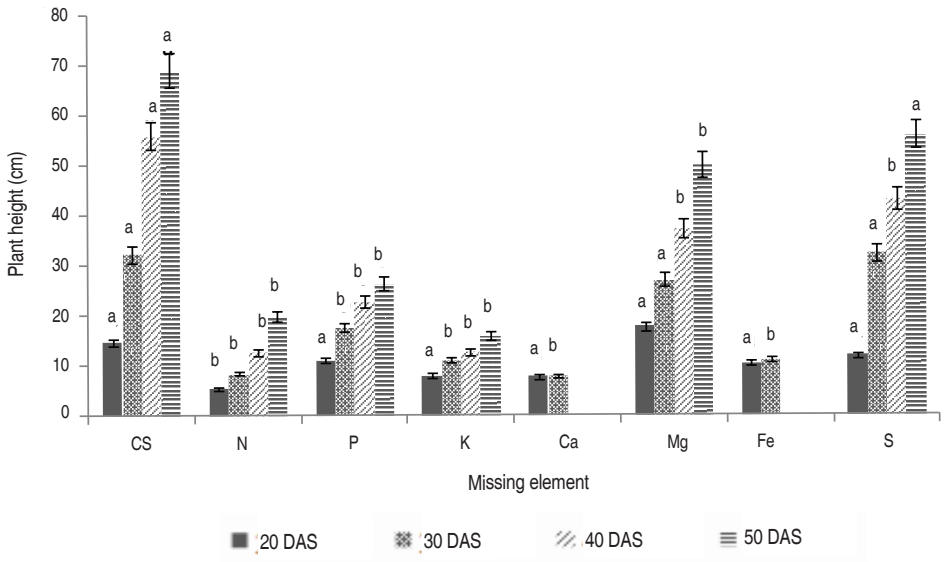

Figure 1. Plant height at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), and sulfur (S). Means (for each date) followed by the same letter do not differ (*P*>0.01).

The omission of nutrients significantly affected the sunflower stem diameter. At 50 DAS, the plants submitted to the omission of N, P and K presented stem diameter 79.4, 73.13 and 69.49% lower than the plants submitted to the complete solution. There was also a significant effect on Ca and Fe omissions, although it was only possible to evaluate these differences up to 30 DAS. In this period, the plants submitted to the solutions with Ca and Fe omission obtained a diameter of 72.06 and 63.51% smaller than that observed in the plants under complete solution. There was no significant effect for treatments containing magnesium and sulfur (Figure 2).

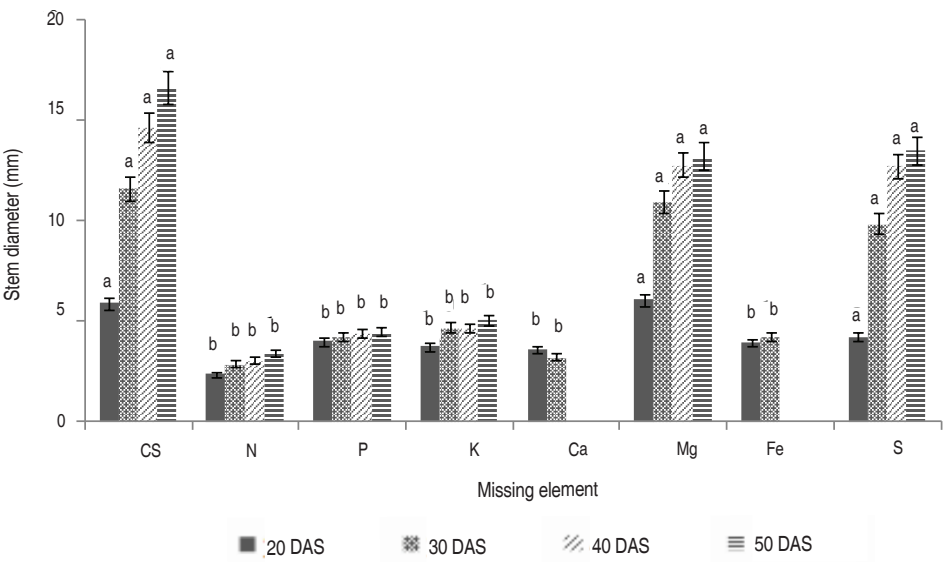

Figure 2. Stem diameter at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of the nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (*P*>0.01).

These results were corroborated by Prado and Leal (2006), Coelho *et al.* (2012), and Gondim *et al*. (2016), who evaluated the stem diameter of sunflower plants, var. Catissol-01, ornamental ginger, and cultivar 1030 maize plants, respectively.

The omission of nutrients, mainly N, P, K, and Ca impacted the stem diameter severely since these are the main responsible for the structural formation of plants (Marschner, 1995). Leaf emission and consequently the sunflower leaf area were strongly affected by the omission of nutrients, with a significant difference when N, P, K, Ca, Fe, and S were omitted (Figure 3).

The absence of these elements in the nutrient solution was responsible for the senescence of the leaves, decreasing their number in the plants. It can be observed in plants submitted to the solution with the omission of

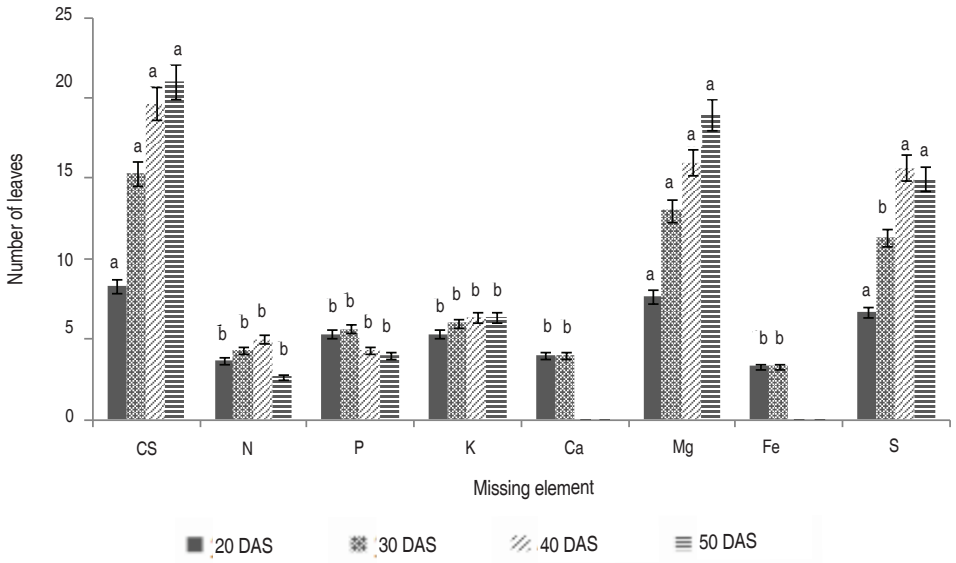

Figure 3. Number of leaves of sunflower plants at 20, 30, 40, and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

N and P which initially had a higher average number of leaves at 20 DAS than the one verified at 50 DAS. At 30 DAS. With Ca, Fe, and S omission there were differences of 73.91, 78.26, and 26.09%, respectively, concerning complete nutrient solution. In the last evaluation, 50 DAS, the differences were 87.3, 80.95, and 9.84%for treatments with the omission of N, P, and K, respectively. The deleterious effects due to the missing elements caused a decrease in the leaf area of sunflower (Figure 4), reducing the surface of light absorption for the photosynthesis as commented by Castro *et al*. (2015).

Probably this reduction occurred due to the low number of leaves, corroborating Maia *et al*. (2014), who stated that the lack of micronutrients in a nutrient solution did not affect the leaf area of plants. The leaf area of plants cultivated with the absence of N, P, K, Ca, and Fe corresponded to 31.89, 43.61, 58.9, 30.22, and 30.57 cm^2, respectively, while the plants that received complete solution reached an average leaf area of 1837.87 cm^2 at 40 DAS.

The omission of N, P, K, Ca, and Fe significantly reduced the dry matter of sunflower plants around 90% in relation to the control (Figure 5A and 5B), corroborating Gondim *et al*. (2016), who observed a reduction in the dry matter of the corn plants, BRS 1030 cultivar, with the deficiency of N, P, K, and Ca in nutritive solution. As previously mentioned, these nutrients are essential for the adequate mineral supply of plants in order to provide normal plant growth. Since there were omissions of these nutrients in the established treatments, the growth of sunflower plants was affected as well as their dry biomass.

Therefore, the omission of N, P, K, Ca, and Fe promoted a restriction on the growth of sunflower plants, with a significant treatment effect on height (Figure 1) stem diameter (Figure 2), number of leaves (Figure 3) and leaf area (Figure 4) of plants.

About the omission of N and K for sunflower lineage LA 1, Cruz *et al.* (1983) observed deficiency symptoms and a significant decrease of the dry matter of the plants relative to the complete nutrient solution. According to Prado and Leal (2006), the individual omissions of N, P, K, and

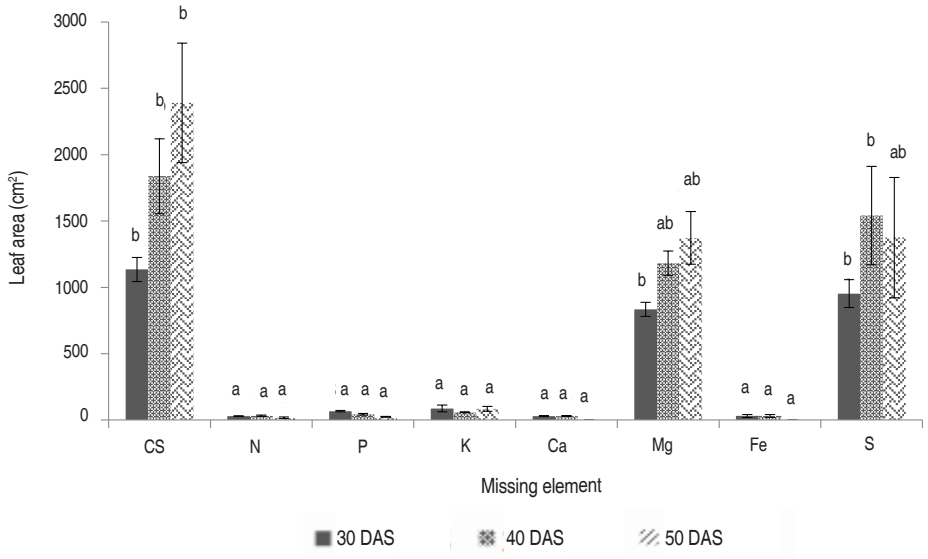

Figure 4. Leaf area of sunflower plants at 20, 30, 40 and 50 DAS cultivated with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

Ca limited the vegetative growth of the sunflower (cv. Catissol-01) and the dry matter produced by the plants. Concerning sulfur omission, there was no effect on the plant height (Figure 1), stem diameter (Figure 2), number of leaves (Figure 3), and leaf area (Figure 4). On the other hand, S omission affected the dry matter yield of sunflower plants (Figure 5), in relation to the control.

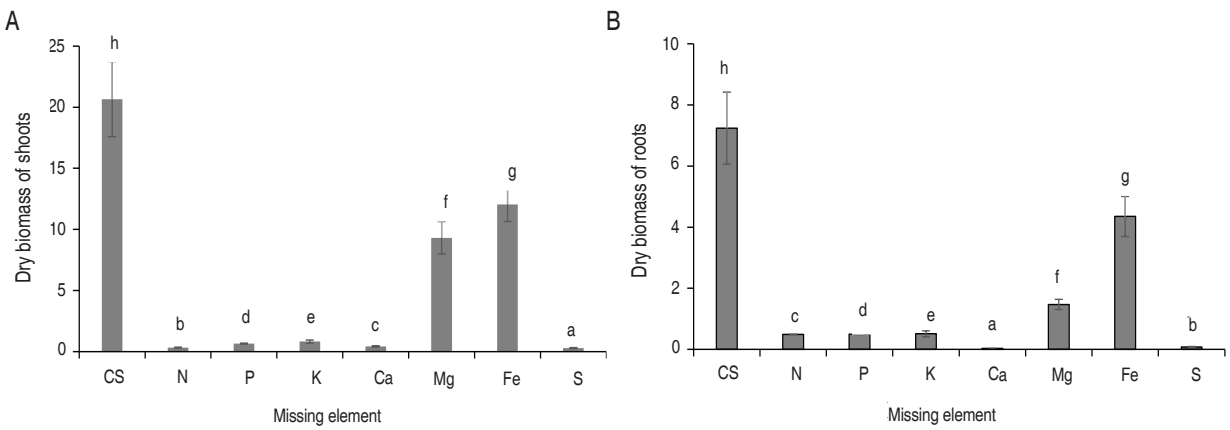

Figure 5. Dry biomass of A. shoots; B. roots of sunflower plants at 50 DAS with complete solution (CS) and with the omission of nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and sulfur (S). Means (for each date) followed by the same letter do not differ (P>0.01).

Description of visual symptoms

The visual deficiency symptoms were initially recorded from DAS by photographs and daily described throughout the experimental period (50 DAS), they are described below.

The absence of nitrogen in the nutrient solution affected significantly the sunflower plants, this was identified at the beginning of the plant growth (20 DAS), uniform chlorosis of the vegetative part of the older leaves, and then reaching all leaves of the plant (Figure 6B), corroborating the findings of Malavolta (2006). According to this author, the nitrogen deficiency in plants results in the collapse of the chloroplasts, occurring a decline in the levels of chlorophyll. Over time, the older leaves were dried from the tip to the ribs and the intense and uniform yellowing reached the younger leaves (Figure 6B).

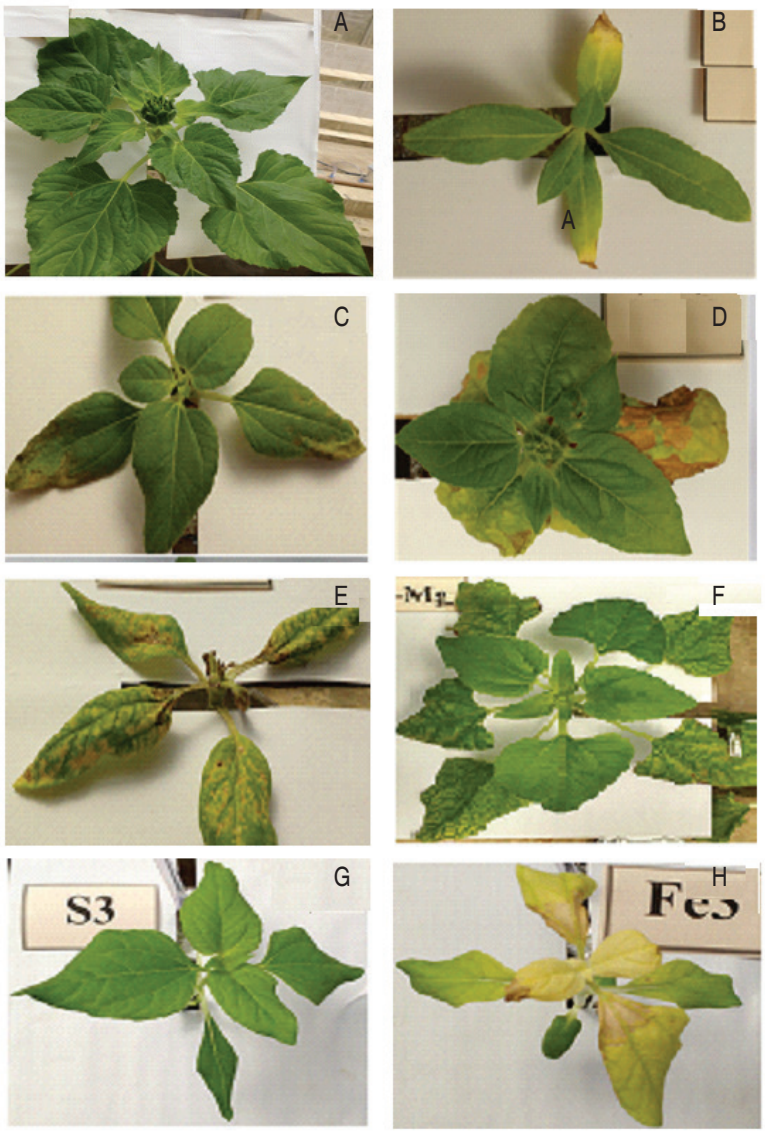

Figure 6. Visual symptoms in sunflower plants cv. BRS-122 cultivated in A. Complete solution; with B. Omission of nitrogen; C. Phosphorus; D. Potassium; E. Calcium; F. Magnesium; G. Sulfur; H. Iron.

According to Epstein and Bloom (2006), the lack of nitrogen causes chlorosis in the leaves, reducing its photosynthetic capacity, the growth rate of the plants, and, in extreme cases, can cause growth paralysis. Therefore, when the N content in the plant is deficient, several physiological processes are compromised and then evolve to visual symptoms of deficiency. Plants under omission of N redistribute via phloem, exhibiting yellow coloration in their older parts (Malavolta *et al*., 1997).

The appearances of brown staining at the edge of the older leaves evolving from the base and from the older leaves to the younger leaves were the visual symptoms of phosphorus deficiency in sunflower plants (Figure 6C). At an advanced stage, the older leaves presented necrosis throughout the leaf edge.

Potassium plays an important role in regulating the osmotic potential of plant cells. It also activates many enzymes involved in respiration and photosynthesis (Taiz and Zeiger, 2009). The symptoms of K deficiency in the sunflower plants were found in older leaves in the form of chlorosis, followed by necrosis of leaf margins and tips. With the intensification of symptoms, it was also observed bending of the youngest leaves (Figure 6D). These symptoms were also observed by Prado and Leal (2006) in research with sunflower.

The Ca omission in the nutrient solution caused chlorosis in the younger leaves, presenting shading in the leaf limbus. Another symptom very characteristic of this element omission is the death of the pointers; and with the continuity of the experiment, the leaves began to show symptoms of necrosis, evolving to the death of the plants. Necrosis in plants can be preceded by generalized chlorosis and bending down the leaves (Figure 6E). Growth can be severely affected if the meristematic regions of the plant die prematurely (Taiz and Zeiger, 2009).

Plants submitted to Mg omission presented visual symptoms of their deficiency: internerval chlorosis, which evolved to bleaching and necrosis of bleached areas, and the old leaves were re-enwrapped and rolled (Figure 6F). Depigmentation is a characteristic symptom of the effects of Mg deficiency since this element is part of the structure of the chlorophyll molecule and its deficiency causes chlorosis (Taiz and Zeiger, 2009). Magnesium is easily redistributed into the plant, so deficiency symptoms usually appear first on older leaves. This pattern of chlorosis occurs because chlorophyll in vascular bundles remains unchanged for more extended periods than chlorophyll in cells between bundles. In severe deficiency, the leaves turn yellowish or white.

The symptoms of sulfur deficiency did not appear at any stage during the 50 experimental days (Figure 6G). However, Malavolta *et al*. (1997) reported that the main characteristic symptom is a yellowing of the younger leaves, which was observed by Cruz *et al*. (1983), cultivating sunflower lineage LA 1 under greenhouse conditions.

The sunflower plants cultivated with nutrient solution without Fe presented reduction in size and chlorosis, initially in the younger leaves (Figure 6H) and then in the medium leaves. Subsequently, the new leaves became completely chlorotic, almost white evolving to necrosis of the leaves (both in the margins as in the surface of the leaves in scattered points). The leaves become chlorotic because iron is required for the synthesis of some chlorophyll-protein complexes in the chloroplast (Taiz and Zeiger, 2009).

CONCLUSIONS

The omission of nitrogen, phosphorus, potassium, calcium, and iron in the nutrient solution severely affected the sunflower plants, preventing their vegetative growth and consequently their development.

The negative interference of the magnesium and sulphur omission in sunflower growth was less harmful than the observed for nitrogen, phosphorus, potassium, and calcium. The absence of nutrients gave clear evidence of the distinct effects that the omission of each element can cause on the visual aspects of sunflower plants.

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REFERENCES

Basseto MA, Ceresini PC and Valério Filho WV.2007. Severidade da mela da soja causada por *Rhizoctonia solani* AG-1 IA em função de doses de potássio. Summa Phytopathologica 33(1): 56-62. doi: 10.1590/S0100-54052007000100008.

Bergmann W. 1992. Nutritional Disorders of Plants. Gustave Fischer, New York. 741 p.

Brandão ZN. 2009. Estimativa da produtividade e estado nutricional da cultura do algodão irrigado via técnicas de sensoriamento remoto. Ph.D.'s Thesis in Natural Resources. Centro de Tecnologia e Recursos Naturais Universidade Federal de Campina Grande, Campina Grande. 152 p.

Carvalho JG, Lopes AS, Brasil E and Júnior RAR. 2001. Diagnose da fertilidade do solo e do estado nutricional de plantas. UFLA/FAEPE, Lavras. 95 p.

Castro ACR, Willadino LG, Loges V, Castro MFA and Aragão FAS. 2015. Macronutrient deficiencies in *Heliconia psittacorum* x *Heliconia spathocircinata* 'Golden Torch'. Revista Ciência Agronômica 46(2): 258-265. doi: 10.5935/1806-6690.20150005

Castro C, Castiglioni VBR, Balla A, Leite RMVBC, Karam D, Mello HC, Guedes LCA and Farias JRB. 1997. Circular Técnica No 13. A cultura do girassol. EMBRAPA-CNPSo, Londrina. 36 p.

Coelho VAT, Rodas CL, Coelho LC, Carvalho JG, Almeida EFA and Figueiredo MA. 2012. Caracterização de sintomas visuais de deficiências de macronutrientes e boro em plantas de gengibre ornamental. Revista Brasileira de Horticultura Ornamental 18(1): 47-55.

Cruz MCP, Ferreira ME and Fernandes NG. 1983. Diagnose por subtração em girassol. Pesquisa Agropecuária Brasileira 18 (12): 1311-1315.

Epstein E and Blomm AJ. 2006. Nutrição mineral de plantas: princípios e perspectivas. Second edition. Planta, Londrina. 403 p.

Ferreira DF. 2011. SISVAR: A Computer Statistical Analysis System. Ciência e Agrotecnologia 35(6): 1039-1042. doi: 10.1590/S1413-70542011000600001

Gondim ARO, Prado RM, Fonseca IM and Alves AU. 2016. Crescimento inicial do milho cultivar brs 1030 sob omissão de nutrientes em solução nutritiva. Revista Ceres 63(5): 706-714. doi: 10.1590/0034-737x201663050016

Hoagland DR and Arnon DI. 1950. Circular 347: The waterculture method for growing plants without soil. First edition. The College of Agriculture, University of California, California. 31 p.

Lira MA, Carvalho HWL, Chagas MCM, Bristot G, Dantas JA and Lima JMP. 2011. Avaliação das potencialidades da cultura do girassol, como alternativa de cultivo no semiárido nordestino. EMPARN, Natal. 40 p.

Maia JTLS, Bonfim FPG, Guanabens REM, Trentin R,Martinez EP, Pereira PRG and Fontes PCR. 2014. Omissão de nutrientes em plantas de pinhão-manso cultivadas em solução nutritiva. Revista Ceres 61(5): 723-731. doi: 10.1590/0034-737X201461050016

Maia JTLS, Guilherme DO, Paulino MAO, Silveira HRO and Fernandes LA. 2011. Efeito da omissão de macro e micronutrientes no crescimento de pinhão-manso. Revista Caatinga 24(2): 174-179.

Malavolta E. 2006. Manual de nutrição mineral de plantas. Agronômica Ceres, São Paulo. 631 p.

Malavolta E, Vitti GC and Oliveira SA. 1997. Avaliação do estado nutricional das plantas: princípios e aplicações. Second edition. Potafos, Piracicaba. 319 p.

Marschner P. 1995. Mineral Nutrition of Higher Plants. Second Edition. Academic Press, New York. 889 p.

Prado RM and Leal RM. 2006. Desordens nutricionais por deficiência em girassol var. Catissol-01. Pesquisa Agropecuária Tropical 36(3): 187-193.

Prado RM, Romualdo LM and Rozane DE. 2007. Omissão de macronutrientes no desenvolvimento e no estado nutricional de plantas de sorgo (cv. BRS 3010) cultivadas em solução nutritiva. Científica 35(2): 122-128.

Rodrigues GC, Carvalho S, Paredes P, Silva FG and Pereira LS. 2010. Relating energy performance and water productivity of sprinkler irrigated maize, wheat and sunflower under limited water availability. Biosystems Engineering 106(2): 195-204. doi: 10.1016/j.biosystemseng.2010.03.011

Taiz L and Zeiger E. 2009. Fisiología vegetal. Fourth edition. Artmed, Porto Alegre. 848 p.

Zobiole LHS. 2010. Marcha de absorção de macronutrientes na cultura do girassol. Revista Brasileira de Ciência do Solo 34(2): 425-433. doi: 10.1590/S0100-06832010000200016

Research article

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Revista Facultad Nacional de Agronomía

Modeling of the immature stages of the species of Noctuidae associated with *Physalis peruviana* L.

UNIVERSIDAD NACIONAL DE COLOMBIA

Modelación de estados inmaduros de especies de Noctuidae asociados a *Physalis peruviana* L.

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Keywords:
Copitarsia decolora
Generalized linear models
Golden berry
Heliothis subflexa
Solanaceae

ABSTRACT

Physalis peruviana L. is currently the second fruit crop more exported of Colombia; however, the pests associated with the culture have been little studied which is important considering that some Noctuidae can cause a decrease of 20% in its production. In this research, the Noctuidae species related to P. peruviana were studied in three farms of La Unión, Antioquia, Colombia. Twelve sampling units, with 30- and 45-day transplanted plants, were distributed throughout the farms and sampled biweekly from March 1st to August 29th of 2014. In the plant canopy and the planted area, immature stages were registered, and statistic models were built in order to describe their trend. The taxonomic identification of adults was made by comparing with the Noctuidae collection of Museo Entomológico Francisco Luis Gallego at Universidad Nacional de Colombia – Sede Medellín, and by using taxonomic keys. Nine Noctuidae species were found in total. Six models were built, four oviposition models for *Agrotis ipsilon* and *Spodoptera* spp., *Copitarsia decolora* and *Heliothis subflexa*, *Megalographa biloba*, and *Peridroma saucia*; a model for larvae and pupae stages was built. The oviposition model for *P. saucia* was the more adjusted, with a Root mean squared predictive difference (RMSPD) of 0.84. The other studied models were suitable to describe the trend of the immature stages; except for *M. biloba* model. This research revealed the ecological characteristics of the Noctuidae species associated with the golden berry crop that affect its productivity.

Palabras clave:
Copitarsia decolora
Modelos lineales generalizados
Uchuva
Heliothis subflexa
Solanaceae

RESUMEN

Physalis peruviana L. es actualmente el segundo cultivo frutícola más exportado de Colombia; sin embargo, las plagas asociadas con el cultivo han sido poco estudiadas, lo cual es importante ya que algunos nóctuidos pueden causar una reducción del 20% en su producción. En esta investigación se estudió las especies de nóctuidos asociadas a *P. peruviana* en tres fincas del municipio de La Unión, Antioquia, Colombia. Doce unidades de muestreo, con plantas entre 30 y 40 días de trasplantadas, fueron distribuidas en las fincas y muestreadas quincenalmente, del primero de marzo al veintinueve de agosto de 2014. En el dosel de la planta y el área plantada se registró el total de estados inmaduros y se construyeron modelos estadísticos con el fin de describir su tendencia. La identificación taxonómica de adultos se hizo por comparación con la colección de Noctuidae del Museo Entomológico Francisco Luis Gallego de la Universidad Nacional de Colombia – Sede Medellín, y mediante el uso de claves taxonómicas. En total se encontraron nueve especies de nóctuidos. Se construyeron seis modelos, cuatro de oviposición para las especies *Agrotis ipsilon* y *Spodoptera* spp, *Copitarsia decolora* y *Heliothis subflexa*, *Peridroma saucia*, y *Megalographa biloba*; uno para larvas y otro para pupas. El modelo de oviposición de *P. saucia* fue el más ajustado con una Diferencia Cuadrática Media de Predicción (RMSPD *por sus siglas en inglés*) de 0,84. Los otros modelos estudiados describen adecuadamente la tendencia de los estados inmaduros, excepto el correspondiente a *M. biloba*. Esta investigación reveló características ecológicas de las especies Noctuidae asociadas al cultivo de uchuva que afectan su productividad.

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In Colombia, *Physalis peruviana* L., known as golden berry, is a fruit of exportation that has had a good reception in the European international market. The crop has increased from 221 cultivated hectares in 1999 (Sanabria, 2005) to more than 1,000 ha in 2009 (Agronet, 2015), and by 2005 it was considered the second more exported fruit after banana (Fischer *et al*., 2005). Nowadays, it has been consolidated as the second most exported fruit product of the country, and in 2016 its total exports were 5.2 million t which represent a value of 23,600 million dollars (López, 2018). Most of the production is obtained by small farmers from the Colombian departments of Cundinamarca, Boyacá and Antioquia (Fischer *et al*., 2014; Zapata *et al*., 2002).

Physalis peruviana (physalis = bladder) have numerous common names according to the country/or regions such as Cape gooseberry (South Africa), Inca berry, Aztec berry, Golden berry, giant ground cherry, African ground cherry, Peruvian ground cherry, Peruvian cherry, pokpok (Madagascar), rasbhari (India), poha aguaymanto poha aguaymanto (Peru), uvilla (Ecuador), uchuva (Colombia), harankash (Egypt), amur en cage (France), and physalis (United Kingdom) (Afsah, 2015). The mechanical, physicochemical, nutritional, and medicinal properties, so as its functionality, associated with this fruit have been described by Puente *et al.* (2011). Its genetic diversity and population structure have been studied by molecular markers (Garzón *et al*., 2015). *Physalis peruviana* is the object of several research in order to take advantage of its secondary metabolites, which exhibit extensive biological properties that could have a potential pharmacological, medicinal, and insecticidal use (Aguirre-Ráquira *et al*., 2014; Cirigliano *et al*., 2008).

However, there have not been developed technological programs or studies for the crop management. Therefore, the losses rise to around 13% (Sanabria, 2005). The studies of insect pests associated with this plant are incipient (Afsah, 2015). In the Noctuidae family, some polyphagous species that affect several organs at different stages of the *P. peruviana* phenology such as *Spodoptera* sp., *Agrotis* sp. and *Feltia* sp., known as cutworms. The larvae is the only harmful stage which can cut the seedling at ground level and can feed on branch, roots, leaves, and shoots (Benavides and Mora, 2005).

The damage caused by these cutworms is not harmful enough to the *P. peruviana* crop, as the damage made by the species that pierce the fruit like *Heliothis subflexa* and *Copitarsia decolora*, the former is considered the most important phytophagous of *P. peruviana* (Zapata *et al*., 2002). The caterpillars pierce the sepals and feed on the fruit in any of its stages, which could reduce the production to 20% according to the climatic condition and the alternative hosts; after the damage, some fruits can fall to the ground or stay in the plant, but in both cases the fruit loses its market value (Benavides and Mora, 2005).

Noctuidae has been the source of many types of researches, with the aim of understanding its behavior, life cycle, and mainly to find ways to control its populations. However, the researches in *P. peruviana* are incipient. The aim of this work was to determine the Noctuidae species associated with *P. peruviana*. The fluctuation of the immature stages according to the development of the plant, and spatial and climatic variables were studied in three farms from the municipality of La Union-Antioquia-Colombia, with the aim of building statistical models to describe the growing trend as a tool for pest management.

Twelve species of Noctuidae were found; cutworm and leaf eaters included *Agrotis ipsilon*, *Megalographa biloba*, *Peridroma saucia* and four species of *Spodoptera* (*S. albula*, *S. eridania*, *S. frugiperda* and *S. ornithogalli*); and two fruit borer, *Copitarsia decolora* and *Heliothis subflexa*. Six models clustering ecological and biological similarities of the species were built, having into account climatic, spatial and temporal variables. Four oviposition models were built for the species *A. ipsilon* and *Spodoptera* spp.; *C. decolora* and *H. subflexa*; *M. biloba*; and, *P. saucia*; besides, larvae and pupae models, without regards of the species. All models, except that of *M. biloba*, are important for predicting purposes in pest management programs.

MATERIALS AND METHODS

Location

The study included 12 sampling units distributed throughout three producer farms of *P. peruviana* in the municipality of La Unión, Antioquia, Colombia. The geographic coordinates of the first farm are 461186.23 m longitude W and 659246.13 m latitude N, the second

farm with 460238.06 m longitude W and 659604.24 m latitude N, and the third farm with 459443.45 m longitude W and, 660349.50 m latitude N, with WGS 84 UTM 18N coordinate system, and 2.509 m mean altitude. The study was performed with plants between 30 and 45 Days After Transplant (DAT) and the sampling were made biweekly, from March 1st to August 29th of 2014. Before the *P. peruviana* was planted, the farms had different covers, which is a critical factor concerning the presence of phytophagous insects and the diseases. The previous cover for the farm 1 was *P. peruviana*, for the farm 2 was stubble low, and for the farm 3 was *Solanum tuberosum*.

Sampling

Every sampling unit corresponded to 10 plants, in each plant the soil under the canopy and the canopy divided into three fractions (top, mid and low) were sampled. The soil under the canopy was flipped to collect the pupae and caterpillars of noctuids. In each fraction of the canopy, the beam, the underside of 20 leaves as the minimum, and the fruits were inspected, looking for the immature stages of noctuids. All the stages were collected in plastic glasses of 16 ounces, covered with a mesh and marked with the number of the plant, the fraction, the metamorphosis stage and the date. The glasses were put in boxes and carried to the laboratory of the Museo Entomológico Francisco Luis Gallego (MEFLG).

Taxonomic identification

The eggs were placed on moistened paper with water until larvae hatching. The larvae were fed daily with leaves of *Ricinus comunis*–until the adult emerged to be identified. The pupae were left in soil moistened with water until the imago emerged. The moths were put in a lethal chamber with ethyl acetate, to be mounted in an entomological pin. The moths were identified by comparing with the Noctuidae collection of the MEFLG with the curator guide (John Albeiro Quiroz-Gamboa), also comparisons with the taxonomic key of Angulo *et al.* (2008) were performed. Besides, the lepidopteran specialist of the United States Department of Agriculture (USDA) (Columbus, Ohio) Steven C. Passoa identified two of the species of larvae from this study.

Statistical analysis

The response variable corresponded to the information of the Noctuidae species that were grouped according to features of the immature stages like pupae, larvae, and oviposition (Table 1). These were correlated with eleven climatic variables, five spatial variables, and a temporal variable. The climatic variables were related to temperature, relative humidity and precipitation, during eight days before sampling, each climatic variable was partitioned according to its features (Table 2). The spatial variable corresponded to the slope and sampling location, north latitude, west longitude, and altitude coordinates. The temporal variable corresponded to the week, correlated with the development phases of the plant as well as the agricultural practices (Table 3). The climatic information was provided by the Instituto de Hidrología, Meteorología y Estudios Ambientales de Colombia (IDEAM), and the spatial information was taken with a Global Position System (GPS) *Garmin e-trx 10®* and analyzed by the software *Arcgis 10.2®*.

Table 1. Response variables of the immature stages of Noctuidae species associated with *Physalis peruviana*.

Type of oviposition				Larvae	Pupae
Gregarious oviposition on the leaf beam (**G*OLB***)	Gregarious oviposition underside of the leaf (***GOUL***)	Isolated oviposition on the leaf beam (***IOLB***)	Isolated oviposition underside of the leaf (***IOUL***)		
P. saucia	*S. albula*	*C. decolora*	*M. biloba*	Larvae	Pupae
	S. eridania	*H. subflexa*			
	S. frugiperda				
	S. ornithogalli				
	A. ipsilon				

Table 2. Explanatory variables: Climatic variables recorded eight days before sampling.

Minimum temperature			Mean temperature			Rainfall		Relative humidity		
Lower	Mean	Higher	Lower	Mean	Higher	Mean	Cumulative	Lower	Mean	Higher
Mitl	*Mitm*	*Mith*	*Metl*	*Metm*	*Meth*	*Rm*	*Rc*	*Rhl*	*Rhm*	*Rhh*

Table 3. Explanatory variables: Spatial and temporal variables.

Explanatory variables					
Slope	X coordinate corrected	Y coordinate corrected	Z coordinate corrected	Sampling unit	Elapsed week
S	*Xc*	*Yc*	*Zc*	*Su*	*Wk*
%	WGS 1984, UTM Zone 18N (m)	WGS 1984, UTM Zone 18N (m)	m.a.s.l.	Distributed throughout three producer farms (1 to 12)	Week of the year

The statistical analysis was made with the Tinn-R® software by using Generalized Linear Models (GLM). GLM is a statistical model for a large family of probability distributions known as the exponential family, that includes important distributions as the Gaussian, Gamma, Chi-squared, Beta, Bernoulli, Binomial and Poisson distributions in order to find statistical differences (Schabenberger and Pierce, 2001). The Poisson distribution was used because the data presented this kind of trend and was of low magnitude. Nevertheless, as there are variables with bigger magnitudes, it was required to get an adjusted model by using Akaike function (AIC) through the step command of Tinn-R®. For the statistical test, it was used the Root Mean Square Predictive Difference (*RMSD*) that provides a higher sameness to validate the prediction model.

Having into account that projected coordinates have a large scale, it was necessary to apply a correction to avoid masking other explanatory variables, as shown below:

$$Cc = (Ci-Cm)$$

Cc: Coordinate corrected

Ci: Coordinates *ith*

Cm: Coordinate minimum

RESULTS AND DISCUSSION

In total, nine Noctuidae species were found. They were distributed in the subfamilies Acronyctinae, Noctuinae, Plusiinae and Cuculiinae. The Acronyctinae included the species: *Spodoptera albula* (Walker, 1857), *S. eridania* (Cramer, 1782), *S. frugiperda* (Abbot and Smith, 1797) and *S. ornithogalli* (Guenée, 1852). These species showed gregarious oviposition covered with the protection of silk and squamae made by the female (Vélez-Ángel, 1997), mainly laid on the leaf underside, in the middle and low fraction of the *P. peruviana* canopy.

The Noctuinae species were *Agrotis ipsilon* (Hüfnagel, 1766), *Heliothis subflexa* (Guenée, 1852) and *Peridroma saucia* (Hübner, 1808). *A. ipsilon* has gregarious oviposition mainly on the leaf underside in the middle and low fraction of *P. peruviana* canopy as well as the *Spodoptera* species. *P. saucia*, except for this species also have oviposition on the leaf beam of *P. peruviana* canopy. Finally, *H. subflexa*, previously classified in Heliothinae (Matthews, 1991), has isolated oviposition mainly on the leaf beam, in the middle and top of the canopy or close to the reproductive organs like branches or fruits of *P. peruviana*.

The only Plussinae species was *Megalographa biloba* (Stephens, 1830), with isolated oviposition mainly on

the leaf underside and in the middle fraction of the *P. peruviana* canopy. The Cuculiinae species found was *Copitarsia decolora* (Guenée, 1852), with isolated oviposition mainly on the leaf beam, in the middle and top of the canopy or close from reproductive organs like branches or fruits of *P. peruviana* as well as *H. subflexa*. *C. decolora* and *H. subflexa* could be considered more important species than the other seven reported in this work because they both feed on the fruits because if the fruit is exported with small larvae and quality flaws could be baned. *C. decolora* and *H. subflexa* were present mainly at the crop reproductive phase, while the other seven species that feed on leaves were present in all the development phases, mainly in the vegetative stage.

The Modelling

The GLM built for each response variable showed the estimated trend for the different immature stages, considering the explanatory variables that more affected the model. Table 4 shows the statistical significance of the variables that describe the model trend and the Akaike index (AIC) that allow inferring if each model is suitable to estimate the trend of the immature stages.

Table 4. Statistical significance of explanatory variables.

Explanatory Variables	β_0	Wk	Su	S	Xc	Yc	Zc	*Mitl*	*Mitm*
Pupae	<0.001	-	<1	-	-	-	-	-	-
Larvae	<1	<0.001	<0.001	<0.1	-	-	.	<0.001	<0.001
H. subflexa and *C. decolora*	<0.001	<0.001	<0.05	-	<0.001	<0.001	-	<0.001	<0.001
P. saucia	<0.05	<0.01|	<0.001	-	<0.01	-	-	-	-
M bibloba	<1	<1	<1	<1	<1	<1	<1	<1	<1
A. ipsilon and *Spodoptera* spp.	<0.001	-	<0.01	<0.001	-	-	<0.05	<0.01	

Explanatory Variables	*Mith*	*Metl*	*Metm*	*Meth*	*Rm*	*Rc*	*Rhl*	AIC	RMSPD
Pupae	-	<0.001	-	-	<0.05	<0.05	-	382.72	1.406
Larvae	-	<0.001	<0.001	<0.001	-	<0.001	<0.001	623.57	2.733
H. subflexa and *C. decolora*	<0.01	<0.001	<0.1	<0.001	<0.05	<0.001	-	953.87	5.414
P. saucia	-	--	<0.01	-	-	<0.001	<1	214.54	0.837
M bibloba	<1	<1	<1	<1	<1	<1	<1	217.25	0.928
A. ipsilon and *Spodoptera* spp.	<0.001			<0.001	-	.	<0.001	422.01	1.714

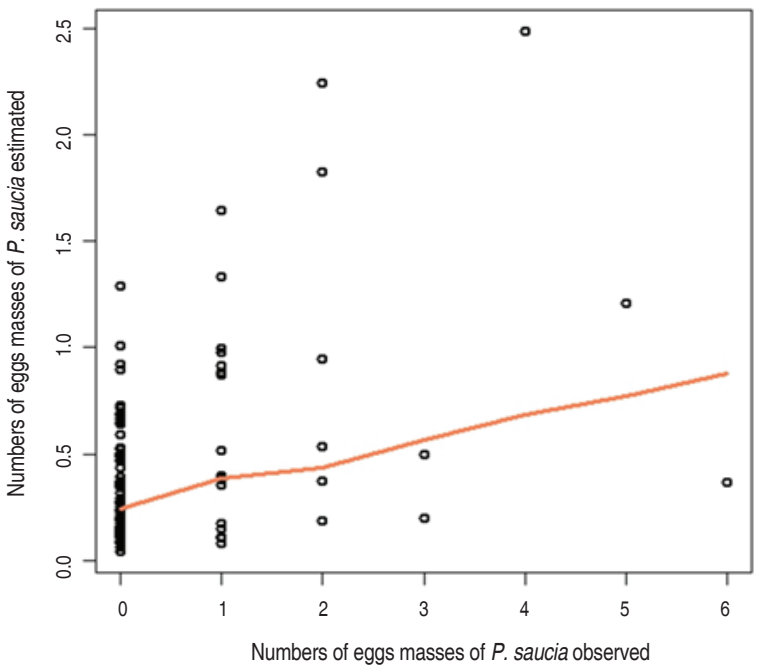
- Without statistical differences.

Before describing each model, it is noteworthy that the most complex model, considering it was affected by 12 of the 17 variables studied, was the oviposition model of *C. decolora* and *H. subflexa* (Table 4). It could be explained because their eggs are laid individual and located from the middle to the upper part of the canopy, therefore they are more exposed and sensitive to most of the variables. On the other hand, the simplest model was the pupae

one, only affected by four variables, suggesting that the states of Noctuidae located underground are less susceptible to most of the variables studied. Besides, it should be highlighted that the *GOLB* model of *P. saucia* oviposition had an index closer to zero.

Peridroma saucia oviposition model

The gregarious oviposition of *P. saucia* on the leaf beam (*GOLB*) in *P. peruviana* showed a model with a *RMSPD* of 0.84, close enough to zero, and all variables had a $P<0.05$, thus the confidence interval was higher than 95%, so it could be used to estimate the oviposition trend of this moth (Figure 1). This kind of model could be used as a tool for integrated pest management, especially if it is complemented with a development model (Choi and Kim, 2014) to predict the time of the larvae first instar, as the proposed for *Ascostis selenaria* (Denis et Schiffermüller) (Lepidoptera: Geometridae). Allowing an effective spraying time, since the egg and the first instar are the most vulnerable to insecticides (Park *et al.*, 2014). The equation suggested that the oviposition of *P. saucia* was influenced negatively by the week (*Wk*) and by the lower relative humidity (*Rhl*).



$$Y' = -16.3308 - (0.0965 Wk) + (1.1886 Metm) + (0.0234 Rc) - (0.0629 Rhl) + (0.3107 S) + (125.8787 Xc) \quad (1)$$

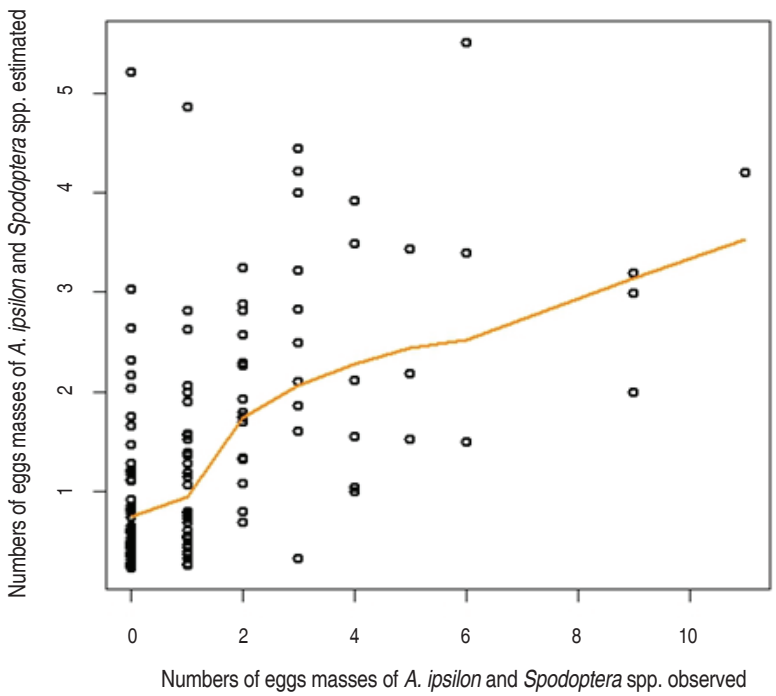
Figure 1. *Peridroma saucia* oviposition model.

The variable *Wk* could be related to *P. peruviana* phenology as well as the agronomic activities because the plant development was matching with chemical pest management to avoid damages in leaves and fruits of *P. peruviana*. The relative humidity and temperature affected the developmental stages of *P. saucia*. The relative humidity and mean temperature were 79.79% and 15.13 °C, respectively, thus the equation model was affected negatively by the lower relative humidity. Moreno-Fajardo and Serna-Cardona (2006b) found that the stages are shorter under a relative humidity of 82.93% and a temperature of 23.97 °C than 65.96% and 17.72 °C. On the other hand, mean temperature (*Metm*), the rain cumulative (*Rc*), the sampling unit (*Su*) and the East corrected (*Ec*) had a positive effect in the oviposition model of *P. saucia*.

Agrotis ipsilon and *Spodoptera* spp. oviposition model

The oviposition model of *A. ipsilon* and *Spodoptera* spp., corresponding to the gregarious oviposition on the leaf underside (*GOUL*) (Figure 2)., had a RMSPD of 1.7142 with a $P<0.05$ for all the variables. It described closely the oviposition trend of the *Spodoptera* species found in *P. peruviana* crop: *S. ornithogalli, S. albula, S, eridania* and *S. frugiperda*, as well as *Agrotis ipsilon.* The oviposition of these species showed similar features because they were found mainly in the mid and low canopy of *P. peruviana*, and the eggs usually were gregarious and covered with silk and flakes (only those of *Spodoptera* spp).

Although this model was not as close to zero as that of *P. saucia* (Figure 1), it could be used to estimate the presence of oviposition of these phytophagous to design a pest management program. The variable affecting the oviposition was the higher value of minimum temperature (*Mith*) with a mean value of 11.8 °C. According to Milano *et al.* (2008), the lowest temperature threshold of *S. frugiperda* is 15 °C and the highest is 35 °C; in both temperatures, the mating frequency is affected. In concordance with Méndez-Barceló (2009) who found that female might laid until 1,000 eggs, but if the temperature reaches 30 °C, the oviposition drops to 386 eggs.



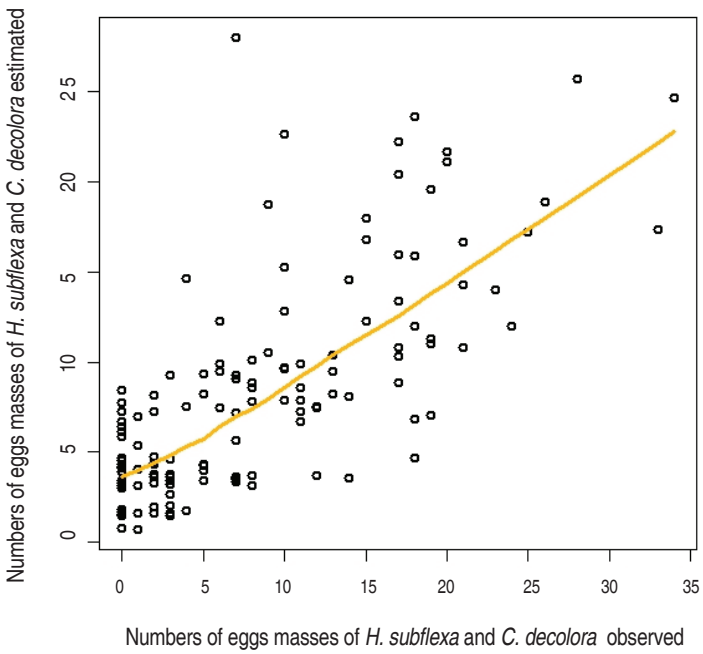
$$Y' = -39.07989 - (0.05482 Su) + (0.06371 Zc) + (2.07891 Meth) + (0.30934 Mitl) - (0.7397 Mith) + (0.14596 Rhl) - (0.05287 S) \quad (2)$$

Figure 2. *Agrotis ipsilon* and *Spodoptera* spp. oviposition model.

Conversely, our model showed that the highest values of mean temperature (*Meth*), with a mean of 16.14 °C and the lowest value of minimum temperature (*Mitl*), with a mean of 8.2 °C had a positive effect in the *Spodoptera* spp. oviposition process. In this case, it is necessary to evaluate the exposition time for every level of temperature, since it may have a lower exposition time in *Meth* and *Mitl*, but for *Mith* had a higher time of exposition. On the other hand, the other variables that affected the model negatively were the slope (*S*) and the sample unit (*Su*), both corresponding to spatial variables. Therefore it can be inferred that there are micro clime and soil variables that are not favorable for the oviposition of *Spodoptera* spp.

Copitarcia decolora and *Heliothis subflexa* oviposition model

The *C. decolora* and *H. subflexa* oviposition model, corresponding to the isolated oviposition on the leaf beam in *P. peruviana* (*IOLB*) (Figure 3), had a RMSPD of 5.414, with all the variables with a $P<0.05$, except for the mean of the mean temperature (*Metm*) with $P<0.1$. The highest value of RMSPD allowed inferring that there may be other variables that can explain better the oviposition behavior of piercing fruit moths; despite the statistical test is supported by a high confidence level in each variable. The GLM (Figure 3) is tight enough to describe the oviposition trend for both species; therefore, it could be used to know the immature stages trend of this species.



$$Y' = -1.189e+01-(3.632e-02Wk)+(5.512e-01Metl)+(9.936e-01Meth)-(1.749e-01Metm)+(5.094e-01Mitl)-(3.546Mith)-(6.945Mitm)-(8.790e-02Rm)+(1.992e-02Rc)-(4{,}268e-02Su)-(1.124e-02Xc)-(2.388e02Yc). \quad (3)$$

Figure 3. *Copitarsia decolora* and *Heliothis subflexa* oviposition model.

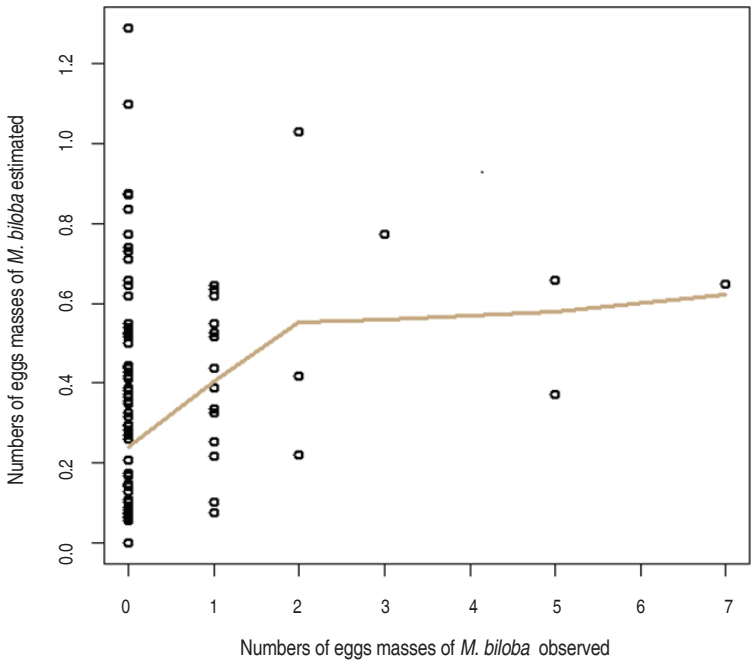
The equation of the model showed that there was a negative effect on the following variables, with $P<0.001$: *Wk*, *Mith*, *Mitm*, and *Ec*. Also, the following variables had a negative impact, with $P<0.05$: *Minth*, *Rm*, *Su*, and *Nc*. Moreover, the variable *Metm* had a negative effect in the model with a $P<0.1$. Four of the variables named correspond to the lower level of temperature because it could be the most important variable. Moreno-Fajardo and Serna-Cardona (2006a) consider that temperature could affect the number of generations in *C. decolora* and probably influence the behavior of this moth, like in *Alabama argillacea* (Lepidoptera: Noctuidae) (Mazza *et al.*, 2006).

On the other hand, the spatial variables *Su* and *Nc*, related to the conditions and location of the sampling units, could be associated with soil and microclimatic conditions. Nevertheless, the lowest temperature minimum registered (*Mitl*), with a mean of 8.02 °C, was positive among the whole variables in this model such as in the *Spodoptera* model, indicating that there could be a relationship between this level of temperature and the behavior of oviposition in both species.

Megalographa biloba oviposition model

The oviposition model of *M. biloba* that corresponded to isolate oviposition under the leaf of *P. peruviana* (*IOUL*) (Figure 4) had a RMSPD of 0.9281; the second model after *P. saucia* with the lowest value. Nevertheless, the P-value for all variables were close to 0.9 (Table 4) which means a confidence interval lower than 10% in

the relationship between the variables studied and the oviposition observed. These results indicated that there is no confidence to estimate the oviposition trend of this species, although Figure 4 showed a good description. The variables studied did not explain the egg trend for *M. biloba* in the *P. peruviana* crop.



$$Y' = -1.144e02 - (7.27e\text{-}01\,Wk) + (7.348e\text{-}02\,Metl) - (5.522\,Meth) + (15.09\,Metm) - (12.99\,Mitl) - (3.686\,Mith) + (22.77\,Mitm) - (8.57\,Rm) + (4.598e\text{-}01\,Rc) - (1.332\,Hrl) - (5.678e\text{-}02\,Su) - (4.115\,Xc) - (9.342\,Yc) - (5.590e\text{-}02\,Zc) - (2.318\ e\text{-}03\ S). \quad (4)$$

Figure 4. *Megalographa biloba* oviposition model.

Larvae model

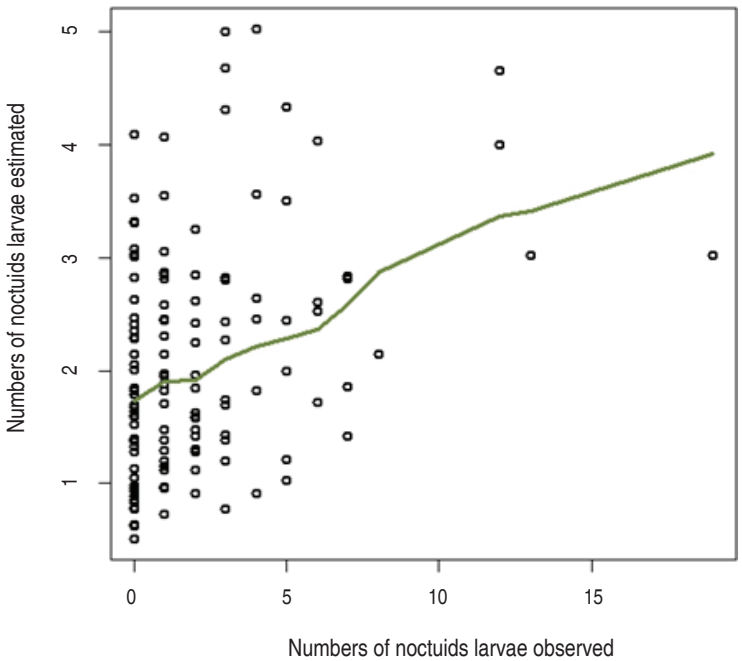
The larvae model of the nine species of noctuids collected in the *P. peruviana* crop had a RMSPD of 2.73252 that is a value relatively far from zero, with a $P<0.05$ for all the variables, except for the slope (*S*) that presented a $P<0.1$. Nonetheless, the model had a good trend description of Noctuidae larvae in the crop (Figure 5), turning it in a pest management tool; therefore, with the different variables evaluated it is possible to define a behavior of the Noctuidae larvae populations in *P. peruviana*.

According to the larvae model equation, the lower value of mean temperature (*Metl*), the mean of mean temperature (*Metm*), the mean of minimum temperature *(Mitm),* the rain cumulative (*Rc*), and the sampling unit (*Su*) had a negative effect on the larvae presence in the field. The temperature had a high incidence in the metamorphosis, while the rain cumulative suggests that soil water accumulation could affect the larvae behavior in the field. Also, the sampling unit is related to microclimatic conditions and is likely that soil features are correlated as well.

Pupae model

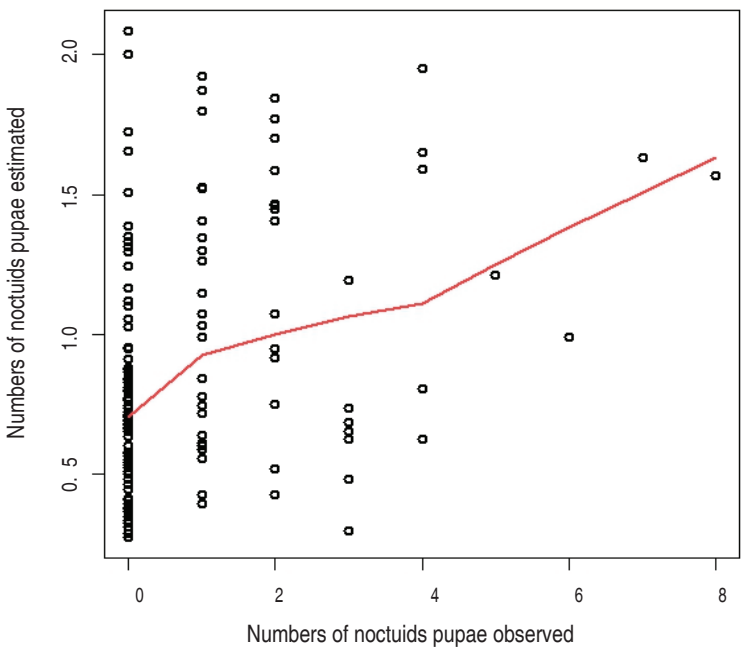
The pupae model of the nine species of noctuids collected in the soil had a RMSPD of 1.4056, which is a value relatively close to zero. The variables had a $P<0.05$, meaning a confidence interval higher to 95%. Therefore, the model had a great trend to describe the pupae of Noctuidae in the crop (Figure 6).

According to the pupae model equation, the mean of mean temperature (*Metm*), the rain cumulative (*Rc*), and the sampling unit (*Su*) had a negative effect in the pupae presence in the field, so as it was observed in the larvae model equation. Considering that the pre-pupae and pupae stages are carried out in the soil, it is important to highlight the possibility that some features of the soil,



$$Y' = -6.518644 + (0.108243 Wk) - (1.04030 Metl) + (1.9953 Meth) - (0.9861 Metm) + (1.523842 Mitl) - (2.0938 Mitm) - (0.021174 Rc) + (0.14205 Rhl) - (0.068996 Su) + (0.015704 S). \quad (5)$$

Figure 5. Larvae model.



$$Y' = 9.54905 - (0.63235 Metm) + (0.14651 Rm) - (0.02591 Rc) - (0.04073 Su) \quad (6)$$

Figure 6. The pupae model.

related to the rain cumulative and the sampling unit, are the reason of the negative effect in this model.

CONCLUSIONS

In most cases, the more important explanatory variables to estimate the trend of noctuids immature stages in the *P. peruviana* crop were the temperature, rainfall cumulative and the elapsed week. The temperature is known as one of the most important variables because it determines the time for the development of the insects' immature stages, considering that they are ectothermic. Therefore, the kind of models built in this work, combined with Grades-Day (used to predict the most important thermal events of the insects), should be considered to reduce its populations, if it is necessary. The cumulative rain dropped the larvae populations and probably reduced the imago activity. Finally, the elapsed week of the crop, another crucial variable, should be considered for insect pest management.

The oviposition found for each noctuid species recorded, as well as each model built, constitutes a tool for integrated pest management. The noctuid oviposition agreed with the feeding preference of each species. Those species that feed on leaves laid their eggs frequently on the canopy middle and low part of the leaves, and those that feed on *P. peruviana* fruit, as *C. decolora* and *H. subflexa*, laid their eggs close to the reproductive organs or on it. These behaviors are important for Noctuidae sampling, monitoring and management.

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REFERENCES

Afsah AFE. 2015. Survey of insects & mite associated Cape gooseberry plants (*Physalis peruviana* L.) and impact of some selected safe materials against the main pests. Annals of Agricultural Science 60(1): 183–191. doi: 10.1016/j.aoas.2015.04.005

Agronet. 2015. Estadísticas; Agrícola: Área, producción, rendimiento y participación. En: Ministerio de Agricultura, http://www.agronet.gov.co/estadistica/Paginas/default.aspx Accessed: November 2015

Aguirre W, Borda D and Hoyos L. 2014. *Potyvirus* affecting uchuva (*Physalis peruviana* L.) in Centro Agropecuario Marengo, Colombia. Agricultural Sciences 5(10): 897-905. doi: 10.4236/as.2014.510097

Angulo A, Olivares T and Weigert G. 2008. Estados Inmaduros de lepidópteros de importancia agrícola y forestal en Chile y claves para su identificación (Lepidoptera: Noctuidae). Third edition. Universidad de Concepción, Concepción, Chile. 154 p.

Benavides M and Mora H. 2005. Los insectos-plaga limitantes en el cultivo de la uchuva y su manejo. pp. 84-95. In: Fischer G, Miranda D, Piedrahita W, Romero J (eds.), Avances en cultivo, poscosecha y exportación de *Physalis peruviana* en Colombia. First edition. Universidad Nacional de Colombia, Unibiblos, Bogotá. 235 p.

Choi KS and Him DS. 2014. Temperature-dependent development of *Ascotis selenaria* (Lepidoptera: Geometridae) and its stage emergence models with field validation. Crop Protection 66: 72-79. doi: 10.1016/j.cropro.2014.08.020

Cirigliano A, Colamarino I, Mareggiani G and Bado S. 2008. Biological effects of *Physalis peruviana* L. (Solanaceae) crude extracts and its major withanolides on *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Boletin de Sanidad Vegetal Plagas 34: 509-515.

Fischer G, Almanza PJ and Miranda D. 2014. Importancia y cultivo de la uchuva (*Physalis peruviana* L.). Revista Brasileira de Fruticultura 36(1): 001-015. doi: 10.1590/0100-2945-441/13

Fischer G, Miranda D and Piedrahita W. 2005. Avances en cultivo, poscosecha y exportación de *Physalis peruviana* en Colombia, primera edición. Universidad Nacional de Colombia, Unibiblos, Bogotá. 235 p.

Garzón GA, Osorio JA, Delgadillo P, Mayorga F, Enciso FE, Landsman D, Mariño L and Barrero LS. 2015. Genetic diversity and population structure in *Physalis peruviana* and related taxa based on InDels and SNPs derived from COSII and IRG markers. Plant Gene 4: 29–37. doi: 10.1016/j.plgene.2015.09.003

López V. 2018. La uchuva en el contexto de la producción agrícola colombiana y los TLC's. Ensayos: Revista de Estudiantes de Administración de Empresas 10 (1): 131-144.

Matthews M. 1991. Classification of the Heliothinae. First edition.Natural Resources Institute, Reino Unido. 198 p.

Mazza SM, Sosa MA, Avanza MM and Bóbeda G. 2006. Comportamiento temporal de plagas del algodonero *(Gossypium hirsutum* L.) en el nordeste argentino. I - Oruga de la hoja (*Alabama argillacea* (Hbn.), Lepidoptera: Noctuidae). In: Memorias XXII Reunión de Comunicaciones Científicas y Tecnológicas 2006. Universidad Nacional de Nordeste, Chaco-Corrientes.

Méndez A. 2009. Influencia alimentaria en la fecundidad de *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) en condiciones artificiales. Anales de Biología 31: 105-108.

Milano P, Filho B, Parra P and Cônsoli L. 2008. Influência da Temperatura na Freqüência de Cóputa de *Anticarsia gemmatalis* Hübner e *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Neotropical Entomology 37(5): 528-535. doi: 10.1590/S1519-566X2008000500005

Moreno OL and Serna FJ. 2006a. Biología de *Copitarsia decolora* (Lepidoptera: Noctuidae: Cuculliinae), en flores cultivadas del híbrido comercial de *Alstroemeria* spp. Revista Facultad Nacional de Agronomía Medellín 59(1): 3257-3270.

Moreno OL and Serna FJ. 2006b. Biología de *Peridroma saucia* (Lepidoptera: Noctuidae: Noctuinae) en flores cultivadas del híbrido comercial de *Alstroemeria* spp. Revista Facultad Nacional de Agronomía Medellín 59(2): 3435-3448.

Park H, Park G and Ahn JJ. 2014. Oviposition model of *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae). Journal of Asia-Pacific Entomology 17(4): 781-786. doi: 10.1016/j.aspen.2014.07.010

Puente A, Pinto CA, Castro ES and Cortés M. 2011. *Physalis peruviana* Linnaeus, the multiple properties of a highly functional fruit: A review. Food Research International 44: 1733–1740. doi: 10.1016/j.foodres.2010.09.034

Sanabria S. 2005. Situación actual del cultivo de la uchuva en Colombia. pp. 1-8. En: Fischer G, Miranda D, Piedrahita W, and Romero J (eds.). Avances en cultivo, poscosecha y exportación de *Physalis peruviana* en Colombia. Universidad Nacional de Colombia, Unibiblos, Bogotá. 235 p.

Schabenberger O and Pierce FJ. 2001. Contemporary statistical models for the plant and soil sciences. First edition. Florida CRC Press, Florida. 760 p.

Vélez R. 1997. Plagas agrícolas de impacto económico en Colombia: Bionomía y manejo integrado. Primera edición. Editorial Universidad de Antioquia, Medellín. 482 p.

Zapata JL, Saldarriaga A, Londoño M and Díaz C. 2002. Manejo del cultivo de la uchuva en Colombia: Boletín Técnico, Corporación Colombiana de Investigación Agropecuaria (Corpoica), Ríonegro. 41 p.

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Effect of nutrient cycle influenced by inter-row cover crops on the nutritional status of rustic grapevine

Efecto del ciclo de nutrientes influenciado por los cultivos de cobertura en el estado nutricional de la vid rústica

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ABSTRACT

The use of plants for permanent or partial coverage of soil in the vineyard inter-rows is a cultural practice used in various wine-growing regions since it is believed that the decomposition of cover crops' straw on the soil surface can increase the availability of nutrients. Therefore, this study aimed to evaluate the nutrient cycling of soil with cover rops in consortium with grapevine (*Vitis labrusca* L. cv. Isabel) cultivated in tropical regions, its nutritional status, and the soil fertility. The experiment was carried out in a vineyard of Isabel cultivar, and three species of ground cover crops were evaluated (*Canavalia ensiformis* L. DC, *Dolichos lablab* L., and weeds). *Canavalia ensiformis* L. DC was more efficient in nutrient accumulation in the canopy than the others. However, the release of nutrients was not statistically different among the cover plants used, being more influenced by the time of grapevine pruning. These coverages did not change the soil chemical properties in the three crop cycles of the two grapevines evaluated and did not affect their nutritional status at the blooming stage of the two harvest seasons evaluated.

Keywords:
Canavalia ensiformis L. DC
Dolichos lablab L
Nutrient release
Vitis labrusca L
Weeds

RESUMEN

El uso de plantas de cobertura parcial o permanente del suelo en las viñas es una práctica cultural utilizada en varias regiones vinícolas, ya que se cree que la descomposición de residuos de plantas de cobertura sobre la superficie del suelo puede aumentar la disponibilidad de nutrientes. Por lo tanto, este trabajo tuvo como objetivo evaluar el ciclo de nutrientes del suelo con cobertura vegetal en consorcio con el cultivo de vid (*Vitis labrusca* L. var. Isabel) cultivada en las regiones tropicales, su estado nutricional y la fertilidad del suelo. El experimento fue realizado en un viñedo de variedad Isabel, y se evaluaron tres especies de cobertura vegetal (*Canavalia ensiformis* L. DC, *Dolichos lablab* L. y malezas). *Canavalia ensiformis* L. DC fue más eficiente en la acumulación de nutrientes en el dosel. Sin embargo, la liberación de nutrientes no fue estadísticamente diferente entre las coberturas vegetales usadas, siendo más influenciada por la época de poda de la vid. Estas no modificaron las propiedades químicas del suelo durante tres ciclos de cultivo de los dos cultivos de vid y tampoco afectaron su estado nutricional en la etapa de floración, en las dos épocas de cosecha evaluadas

Palabras clave:
Canavalia ensiformis L. DC
Dolichos lablab L
Liberación de nutrientes
Vitis labrusca L
Malezas

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The use of plants for permanent or partial coverage of soil in the vineyards is a cultural practice used in various wine-growing regions. This technique consists in use plants in consortium with grapevine soil, let them complete their cycle and then convert them in in straw deposited on the soil surface, slightly crushing or incorporating them, with the aid of a roller-crimper (Nachtigal and Schneider, 2007). The cover crop protects the soil from climatic agents and can also maintain or increase the level of soil organic matter, mobilize and cycle nutrients as well as favor the soil biological activity (Guerra and Teixeira, 1997; Fourie, 2012). Furthermore, according to Suzuki and Alves (2006) and Ferreira *et al*. (2012), cover crops significantly contribute to improving the physical properties of soil, increasing the water storage capacity and allowing a nutritional balance for the succeeding of crops.

The use of cover crops on soil can reduce the application of conventional fertilizers and herbicide in vineyards (Souza *et al*., 2012). When soils are tilled and exposed to the intense use of herbicides for weed control, they are prone to nutrient leaching. This practice leads to successive re-applications of chemical fertilizers; consequently, this management also increases production costs and environmental contamination risks (Teixeira *et al*., 2011).

The decomposition of cover crops' straw on the soil surface can increase the availability of nutrients, favoring their absorption by the grapevine. Especially, nitrates (N-NO_3), which is the N form absorbed in higher quantities by the fine roots of the grapevine that presents rapid growth when it blooms (Eissenstat, 2007). Zalamena *et al*. (2013) found higher nitrogen (N) content in the leave collected in full bloom when worked with vineyards intercropped with buckwheat, white oat, and ryegrass. However, the cover crop management via mowing and transferring the crop straw from the inter-row to the grapevine row decreased N content in the leave collected in full bloom. In studies with Fabaceae jack bean (*C. ensiformes* L. DC) and crotalaria (*Crotalaria juncea* L.), Faria *et al*. (2004) found improvements in soil chemical properties, increasing the levels of soil organic matter, exchangeable calcium (Ca), and the Cation Exchange Capacity (CEC) value. The beneficial effect of the lablab on the soil chemical characteristics was restricted to the upper soil layer (0-10 cm deep).

According to Crusciol *et al*. (2008) and Giongo *et al*. (2011), the nutrient release from mixed cover crops depends on several factors: interaction between the species used, biomass management, plant sowing and cutting time, chemical composition of plant residues and its C/N relation, and soil and weather conditions. Thus, the factors that regulate the decomposition can play an important role in crop management, enabling the development of farming techniques that improve the utilization of nutrients in plant residues (Gama-Rodrigues *et al*., 2007).

Regarding the use of cover crops, Gama-Rodrigues *et al*. (2007) stated that the use of legumes is a strategy to enhance sustainability, benefiting the soil, the environment of economically important crops. Therefore, this study aimed to evaluate the nutrient cycling of soil with cover crops in consortium with grapevine (Isabel cultivar) cultivated in tropical regions, its nutritional status, and the soil fertility.

MATERIALS AND METHODS

Studied area

The experiment was conducted in the municipality of Itapuranga, Goiás, at the Capoeira Grande Farm (15°34'32"S, 50°00'31"W) with an average altitude of 635 m.a.s.l. The climatic conditions in the region are a rainy season from October to April, with an average rainfall of 1600 mm, and average temperatures of 27 and 34 °C in the dry and wet season, respectively.

The climatic data of that region was considered for the development of this study (Figure 1). The meteorological data, from 2013 to early 2014, were obtained from the automatic station of the City of Goiás, located at 47 km from the experimentation site.

The soil of the experimental area was classified as Red Latosol (Santos, 2013) similar to Oxisol (Soil Survey Staff, 1999). The experimental area was formed by irrigated vineyard (small sprinklers), with cv. Isabel grafted on IAC 572 'Jales' grapevine rootstock, in trellis type conduction system spaced 2.5×2.5 m. At the time of the experiment development, the vineyard was two years old after grafting.

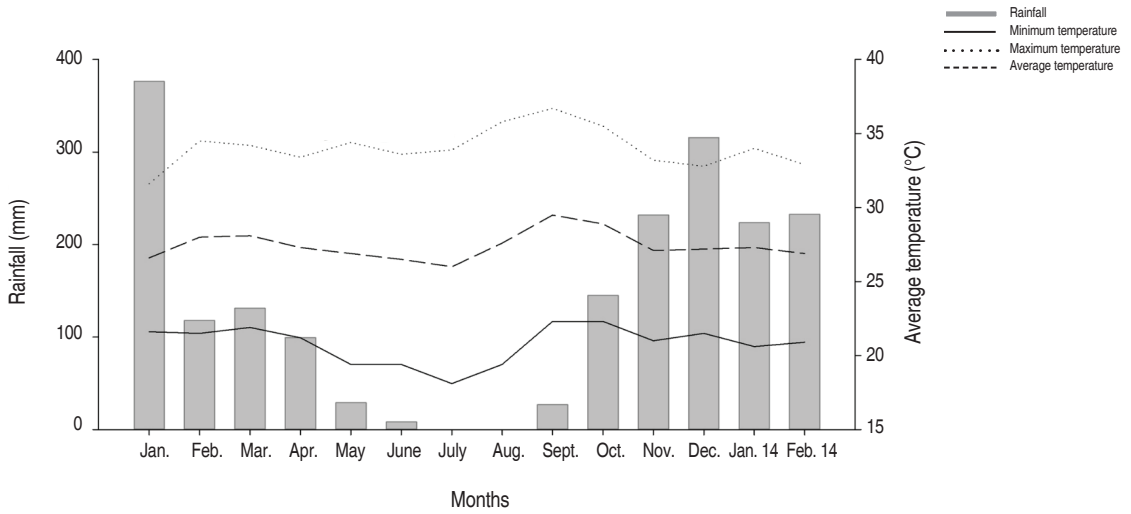

Figure 1. Monthly weather data: Total rainfall and average highest and lowest temperature, obtained from the automatic weather station (INMET, 2014).

Experiment design and treatments

The experiment consisted of a randomized blocks design of six treatments established in a factorial arrange (3×2) with five repetitions. The first factor was cover plants species: *Canavalia ensiformis* L. DC, *Dolichos lablab* L. (lablab) and weeds. The second factor was two different pruning times in the vineyards, performed based on cover crop seeding time. That is, the first grapevine pruning season began 25 days after cover crops sowing (DAS) and the second at 55 DAS. Each plot 9 m² (2.0×4.5 m) was contained two grapevine plants.

Period of conduction

The experiment was conducted in two growing seasons. The period called "winter season" started in February and ended in August 2013, and the period called the "summer season" was from August 2013 to February 2014. In the winter season, the grapevine pruning was performed on March 2nd, 2013 (first pruning, 25 DAS) and April 1st, 2013 (second pruning, 55 DAS) employing a long pruner, keeping five gems per grapevine stick. During the summer season, the grapevine pruning was held on August 31st (first pruning, 25 DAS) and September 30th, 2013 (second pruning, 55 DAS) employing a short pruner, keeping two gems per grapevine stick. After each pruning, bud dormancy breaking was conducted with hydrogenated cyanamide (5%), applied with a foam roller.

Three cycles of cover crops were evaluated: (i) sowing was done on February 5th, 2013, (ii) the plants regrowth was evaluated after their management (mowing April 6th, 2013), and (iii) a new sowing was conducted on August 6th, 2013; monitoring their development within sixty days after sowing. Before each sowing, chemical control was conducted for existing weeds in all plots, using 3 L ha^{-1} glyphosate. Sowing was done in furrows spaced 0.45 m and approximately 1 to 2 cm deep, performed manually, using five seeds of *C. ensiformis* and ten seeds of lablab, without any fertilization or seed inoculation. Weeds emerged from the soil seed bank.

The plots composed of the weed cover showed the following species in the first cycle: *Bidens pilosa* L. > *Digitaria horizontalis* Willd. > *Euphorbia heterophylla* L. > *Commelina benghalensis* L. > *Siegesbeckia orientalis* L. Weeds in the third cycle were: *Bidens pilosa* L.> *Digitaria horizontalis* Willd. > *Amaranthus retroflexus* L. > *Sida rhombifolia* L. > *Euphorbia heterophylla* L. > *Commelina benghalensis* L. The weeds in the second cycle were not determined.

Crop fertilization was scheduled during the winter and summer seasons (Table 1). In order to manage vineyard health in winter, products containing Metiram + Pyraclostrobin and Metalaxyl-M + Mancozeb and in the

summer were sprayed with Metalaxyl-M + Mancozeb, Thiophanate-Methyl and Chlorothalonil + Azoxystrobin + Difenoconazole to prevent and control fungal diseases.

Nutritional status evaluation of cover crops

The aerial part of biomass samples was dried at 65 °C to determine cover crops' nutrients at the time of the cuts. The method for chemical analysis was wet digestion of the dry samples according to the methodology of Bataglia *et al.* (1983). Traces elements (N, P, K, Ca, Mg, Cu, Fe, Mn, and Zn) were determined in the aerial part of cover crops, reporting the total accumulation of traces as kg ha^{-1}.

Table 1. Fertilizer application, in grams per grapevine plant, using nitrogen (N), phosphorus pentaoxide (P_2O_5), potassium oxide (K_2O) and micronutrient during winter and summer seasons.

Application time		Winter season				Summer season			
		10 DBP	15 DAP	45 DAP	80 DAP	10 DBP	15 DAP	45 DAP	80 DAP
Fertilizer (g $plant^{-1}$)	P_2O_5	35	-	-	-	60	-	-	-
	N	-	20	20	-	8	20	20	-
	K_2O	-	-	-	15	20	-	-	15
	FTE BR12	-	25	-	-	-	-	-	-

DBP = Days before pruning; DAP = Days after pruning; FTE = Fritted Trace Elements.

Evaluation of nutrient release by cover crops

'Litter bags' were used to evaluate the plant decomposition (Thomas and Asakawa, 1993). Four litter bags were placed randomly on the soil surface of each plot during the three cycles of cover crops. Sampling was performed at 20, 40, 60 and 80 d, and in each sampling, the litter bags were oven-dried at 65 °C until reaching a constant weight.

The parameters associated with the nutrient release dynamics were calculated based on the weight of dry residue remaining after 80 d of decay and the nutrient concentration in them. To describe the nutrient release from the plant straw, the exponential mathematical model $X=X_0^{(-kt)}$ were adopted, where X is the amount of remaining nutrient that was presented after a time t (d), X_0 is the initial amount of nutrient, and k is a release constant (Thomas and Asakawa, 1993). By reorganizing the equation terms, it is possible to calculate the release constant of nutrients (k) by the material, $k=-\ln (X/X_0)/t$. With the value of k, the half-life ($t_{1/2}$) at which half of the nutrients contained in the residue will be released was calculated ($T_{1/2}$ =0.693/k) (Paul and Clark, 1989).

Soil fertility evaluation

At the end of 2013 (summer season), four soil sub-samples were collected in each plot, with the help of a Dutch auger, to form a composite sample from the 0-0.20 m layer. In the laboratory, the organic matter (OM), pH, Cation Exchange Capacity (CEC), P, K, Ca, Mg, Al and potential acidity (H+Al) were determined, using the methodology of Embrapa (1997).

Grapevine nutritional status evaluation

The leaf nutritional diagnosis was made using grapevine leave at two different pruning times in each evaluated season. The winter sampling was held on March 31st and April 30th of 2013, counting as first pruning and second pruning, respectively. The summer season sampling was held on October 5th and 27th, first and second pruning, respectively. For the analysis, five full leave per plant were collected in each plot, totaling ten leave per sample. The leave were collected in the grapevine's full bloom stage, opposite to the first bunch of the season's branch. The leave were washed and dried in a forced-air oven at 65 °C until reaching constant weight; they were milled and prepared for the analysis of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), according to the methodology of Bataglia *et al.* (1983).

Table 2. Nutrient levels in gravepine leave collected during blooming.

Nutrient	Deficiency	Slight deficiency	Normal	Slight excess	Excess
			(g kg^{-1})		
N	< 26	26-29	30-35	36-40	>40
P	< 1.3	1.3-2.3	2.4-2.9	3.0-3.9	>3.9
K	< 7	7-14	15-20	21-29	>29
Ca	< 8	8-12	13-18	19-32	>32
Mg	< 3.0	3.0-4.7	4.8-5.3	5.4-10.0	>10.0
S	< 2.0	2.0-3.2	3.3-3.8	3.9-6.0	>6.0
			(mg kg^{-1})		
B	< 20	20-44	45-53	54-100	>100
Cu	< 5	5-17	18-22	23-40	>40
Fe	< 50	50-96	97-105	106-200	>200
Mn	< 20	20-66	67-73	74-300	>300
Zn	< 1.5	15-29	30-35	36-200	>200

Source: Terra and Tecchio (2008).

The concentration levels recommended by Campinas Agronomic Institute were used for the vineyard leaf nutritional diagnosis (Table 2) (Terra and Tecchio, 2008).

The collected data were submitted to ANOVA, and the means were compared using Tukey test at a 5% level.

RESULTS AND DISCUSSION

Nutritional status of cover crops

The *C. ensiformis* biomass showed a higher accumulation of N in the first cycle, and P and K in the second cycle (Table 3). Padovan *et al*. (2011) found that *C. ensiformis* was efficient in cycling nutrients, especially immobilized N, K and Ca. It was also determined that *C. ensiformis* can accumulate N, K and Ca in concentrations of 415, 256 and 327 kg ha^{-1}, respectively, when evaluating extract nutrient capability from the soil through organic system production in the summer conditions (Saminêz *et al*., 2006). These results reinforce its great potential as a cover crop and provide basic information for planning the management of plant biomass. Bertin *et al*. (2005) found higher total nitrogen content in Crotalaria, *C. ensiformis*, and lablab, statistically differing from fallow and millet. This result confirms the relationship established by De-Polli and Chada (1989) in which the N content is superior in more tender species.

Lablab did not present statistical difference regarding N accumulation in the first cycle and P and K in the second cycle. Given these results, it is possible to infer that the weeds have benefited from good soil fertility, justifying by the efficient accumulation of nutrients in their biomass (Table 3). In a study of cover crops developed in Cerrado region (State of Maranhão), it was found that lower release of N by the spontaneous vegetation was probably due to the low amount of N in the residue, associated with the low dry plant matter decomposition rate, they also found lower accumulation of P in the biomass (Leite *et al*., 2010).

Silva *et al*. (2002) found, in orange orchard, that *C. ensiformis* and the lablab were the species that showed higher amount of macronutrient levels in the canopy, followed by velvet bean that stood out in N and P levels, *Crotalaria spectabilis* for K and Ca, and dwarf velvet bean for N and S. According to Souza *et al*. (2012), the content and amount of nutrient uptake by the cover crops species can influence the

decomposition of plant material and the performance of the subsequent cultivation. In several studies involving cover crops, the amount of accumulated nutrients depends on the species, the phenological management stage, and the climatic conditions. Regarding the micronutrient accumulation, a significant effect was observed for the canopy corresponding to Cu and Zn levels, the weeds accumulated the lowest amount of these micronutrients, and *C. ensiformis* presented the highest levels, not significantly differing from lablab (Table 3).

Table 3. Nutrient accumulation in the canopy of cover crops, lablab (LB), *C. ensiformis* (CE) and weeds (W) in three consortium cycles and two grapevine pruning times.

Nutrients	Cycle	Grapevine pruning time		F	Treatments			SMD	CV (%)
		1st	2nd		LB	CE	W		
		(kg ha^{-1})			(kg ha^{-1})				
N	1°	188.42	163.93	1.58^{ns}	158.06 b	253.51 a	116.95 b	59.54	30.28
	2°	154.64	181.19	1.69^{ns}	167.54	193.38	142.82	62.41	33.30
	3°	234.73	256.37	0.29^{ns}	253.38	252.59	230.68	121.36	44.27
P	1°	28.74	22.77	4.30*	22.55 a	27.77 a	26.94	8.79	30.57
	2°	26.24	23.69	0.89^{ns}	21.55 b	33.43 a	19.92 b	8.27	29.70
	3°	28.92	26.69	0.30^{ns}	27.45	31.12	24.85	12.34	39.77
K	1°	64.66	46.58	7.55*	52.80	56.83	57.24	20.10	32.38
	2°	52.07	57.26	0.81^{ns}	53.60 ab	65.23 a	45.17 b	17.59	28.84
	3°	58.40	53.93	0.21^{ns}	55.23	56.70	56. 55	30.00	47.85
Ca	1°	72.53	60.63	0.85^{ns}	60.17	62.31	77.27	39.49	53.14
	2°	122.27	139.45	0.69^{ns}	151.56	138.08	102.94	63.06	43.17
	3°	87.29	78.00	0.49^{ns}	83.48	87.16	77.31	40.54	43.95
Mg	1°	16.36	11.65	6.03*	12.92	14.18	14.92	5.86	37.48
	2°	16.44	17.56	0.34^{ns}	16.70	19.59	14.71	5.83	30.75
	3°	8.83	11.09	0.69^{ns}	8.83	11.80	9.24	8.32	28.96
Cu	1°	0.56	0.53	0.07^{ns}	0.52	0.55	0.57	0.32	52.05
	2°	0.86	0.83	0.13^{ns}	0.79 ab	1.02 a	0.73 b	0.27	28.85
	3°	0.29	0.36	1.40^{ns}	0.26	0.34	0.37	0.18	48.94
Fe	1°	15.20	11.44	4.65*	13.45	13.90	12.60	5.33	35.86
	2°	20.81	22.33	0.19^{ns}	25.55	23.98	15.18	10.54	43.78
	3°	6.62	6.52	0.01^{ns}	6.36	7.50	5.85	2.76	37.67
Mn	1°	3.85	5.20	1.67^{ns}	4.10	4.33	5.15	3.18	62.87
	2°	5.62	5.57	0.003^{ns}	5.14	6.74	4.91	2.93	46.95
	3°	4.28	5.03	0.26^{ns}	4.08	5.35	4.53	4.51	37.53
Zn	1°	0.78	0.96	1.29^{ns}	0.80	0.97	0.83	0.46	47.40
	2°	1.64	1.40	1.71^{ns}	1.39 ab	1.91 a	1.26 b	0.56	32.96
	3°	0.72	0.70	0.05^{ns}	0.70	0.72	0.72	0.33	42.15

Means followed by different letters in the same line differ according to the Tukey test to 5% level. ^{ns}not significant (P>0.05), *significant (P<0.05), **significant (P<0.01). SMD=Significant Mean difference; CV=Coefficient of variation.

Copper is an essential element for plants, but in high concentrations can cause toxicity, which may extend to man and animals that consume copper contaminated food. Cover crops can be an alternative for the mitigation of copper excess in the soil, which by decomposing provide an increase of the straw amount, among which there is the organic material capable of promoting the immobilization of the available copper and decrease its presence in soil (Albarello *et al*., 2013).

Cavalcante *et al*. (2012) found that the plants evaluated for cover crops had, among the micronutrients, high accumulation of Fe and Mn. Duarte and Coelho (2008) observed that the legumes *Crotalaria* sp, *C. ensiformis* and velvet bean (*Mucuna* sp) extracted higher amounts of P, Ca, Mg, S, Zn, and Fe than weeds. In this study, the levels of P, K, Ca, and Fe were higher in the plants grown in the first pruning time and the first cover crop cycle than the other two cycles.

Release of nutrients by cover crops

The nutrient release parameters are not shown for Mn in the first cycle, and Fe in the three cycles because their contents were higher than the initial after 80 d of decomposition, indicating that sample contamination might have occurred by soil residues. The N, P, K, and Mg release parameters were significantly higher in the first pruning time, showing a higher amount of nutrients released, constant decomposition and shorter half-life (Table 4).

Table 4. Quantity released (kg ha^{-1}), release constant k (d^{-1}) and half-life ($t_{1/2}$) for nutrients of cover crops, lablab (LB), *C. ensiformis* (CE) and weeds (W), after 80 d in decomposition in the first consortium cycle and two grapevine pruning times.

Nutrient	Parameter	Pruning time		F	Treatments			SMD	CV (%)
		1st	2nd		LB	CE	W		
					1st cycle				
N	(kg ha^{-1})	189.33	119.99	7.44*	144.25	140.92	178.81	78.80	45.01
	k (d^{-1})	0.030	0.022	20.44**	0.025	0.027	0.026	0.006	19.84
	$t_{1/2}$ (d)	23.67	33.16	16.31**	28.65	26.58	30.02	7.28	22.63
P	(kg ha^{-1})	24.98	17.13	5.85*	18.28	23.71	21.17	10.06	42.22
	k (d^{-1})	0.026	0.018	6.08*	0.022	0.025	0.020	0.009	36.27
	$t_{1/2}$ (d)	28.25	46.94	5.21*	37.37	31.48	43.93	25.35	59.57
K	(kg ha^{-1})	62.05	43.13	8.77**	50.29	54.12	53.36	19.79	33.25
	k (d^{-1})	0.040	0.033	13.60**	0.038	0.038	0.034	0.006	15.80
	$t_{1/2}$ (d)	17.54	21.72	12.61**	18.74	18.99	21.17	3.64	16.40
Ca	(kg ha^{-1})	47.97	34.08	1.55ns	36.41	41.05	45.62	34.54	42.23
	k (d^{-1})	0.014	0.010	4.07ns	0.011	0.013	0.012	0.007	30.62
	$t_{1/2}$ (d)	51.84	56.88	0.91ns	58.41	53.21	51.46	16.36	26.59
Mg	(kg ha^{-1})	14.67	9.53	7.94*	11.28	12.62	12.40	5.65	41.28
	k (d^{-1})	0.029	0.022	11.12**	0.026	0.028	0.022	0.006	22.33
	$t_{1/2}$ (d)	25.20	34.09	14.32**	28.17	27.07	33.70	7.28	21.71
Cu	(kg ha^{-1})	0.49	0.47	0.06ns	0.46	0.49	0.48	0.33	29.76
	k (d^{-1})	0.028	0.026	0.36ns	0.028	0.029	0.023	0.010	33.39
	$t_{1/2}$ (d)	26.98	29.92	0.69ns	25.95	26.20	33.19	11.00	34.16
Zn	(kg ha^{-1})	0.59	0.70	0.43ns	0.61	0.78	0.49	0.49	31.38
	k (d^{-1})	0.018	0.016	0.71ns	0.0168 ab	0.0203 a	0.0129 b	0.007	34.88
	$t_{1/2}$ (d)	44.37	51.28	0.93ns	45.94 ab	35.11 b	62.42 a	22.15	40.92

Means followed by different letters in the same line differ according to the Tukey test to a 5% level. nsnot significant (P>0.05), *significant (P<0.05), **significant (P<0.01). SMD=Significant Mean difference; CV=Coefficient of variation.

In the first cover crop cycle, there was a significant difference in the release constant (k) and half-life of Zn, especially in the *C. ensiformis,* that presented the highest release constant (0.0203 d^{-1}) of this nutrient and shorter half-life (35.11 d), but not differing from the lablab, which in turn does not differ from weeds (Table 4).

In the second cycle, there was a significant difference for the amount released and release constant for N, P, K, Ca, Cu, and Zn; the half-life was significant for N, P, K, and Ca (Table 5). Generally, lablab performed better in the release of nutrients for Ca and Zn. The release of P was higher for *C. ensiformis* (24.99 kg ha^{-1}), with a half-life of 43.46 d, revealing its efficiency in recycling this nutrient because it also showed higher dry P in the biomass (Table 5). Gamma-Rodrigues *et al*. (2007) found that N, P, Ca, and Mg release rates were higher in the *C. ensiformis* compared to *Arachis* sp, siratro, tropical kudzu, and weeds. Calonego *et al*. (2012) also found that the lablab straw was efficient in N, P, and K release.

Table 5. Quantity released (kg ha^{-1}), release constant k (d^{-1}) and half-life ($t_{1/2}$) for nutrients of cover crops, lablab (LB), *C. ensiformis* (CE) and weeds (W), after 80 d in decomposition in the second consortium cycle and two grapevine pruning times.

Nutrient	Parameter	Pruning time		F	Treatments			SMD	CV (%)
		1st	2nd		LB	CE	W		
					2ndcycle				
N	(kg ha^{-1})	115.51	130.63	0.47^{ns}	122.87	156.51	89.83	67.93	48.76
	k (d^{-1})	0.017	0.017	0.0001^{ns}	0.0163 ab	0.0211 a	0.0124 b	0.006	33.49
	$t_{1/2}$ (d)	45.86	52.19	1.05^{ns}	49.88 ab	34.78 b	62.41 a	19.13	34.48
P	(kg ha^{-1})	16.89	15.37	0.31^{ns}	12.84 b	24.99 a	10.56 b	8.40	46.01
	k (d^{-1})	0.012	0.014	0.63^{ns}	0.0115 b	0.0177 a	0.0100 b	0.005	31.06
	$t_{1/2}$ (d)	59.13	63.18	0.48^{ns}	66.55 a	43.46 b	73.44 a	18.15	26.23
K	(kg ha^{-1})	39.88	42.06	0.11^{ns}	39.99 ab	53.42 a	29.51 b	20.62	44.47
	k (d^{-1})	0.018	0.017	0.18^{ns}	0.0170 ab	0.0219 a	0.0133 b	0.006	29.85
	$t_{1/2}$ (d)	41.20	49.33	2.88^{ns}	45.98 ab	34.91 b	54.91 a	14.82	28.92
Ca	(kg ha^{-1})	70.37	94.17	2.02^{ns}	97.08	97.18	52.53	51.85	24.05
	k (d^{-1})	0.011	0.014	0.36^{ns}	0.0128 ab	0.0153 a	0.0090 b	0.005	38.30
	$t_{1/2}$ (d)	68.92	64.44	0.47^{ns}	62.64 ab	54.71 b	82.69 a	20.34	26.95
Mg	(kg ha^{-1})	10.71	13.58	2.09^{ns}	11.87	14.81	9.76	6.15	44.73
	k (d^{-1})	0.013	0.004	8.16**	0.016	0.019	0.014	0.006	33.72
	$t_{1/2}$ (d)	57.31	43.57	6.26*	47.94	47.31	56.08	17.01	29.79
Cu	(kg ha^{-1})	0.47	0.54	0.68^{ns}	0.456 ab	0.684 a	0.381 b	0.26	45.94
	k (d^{-1})	0.010	0.014	6.01*	0.0107 ab	0.0148 a	0.0093 b	0.005	38.40
	$t_{1/2}$ (d)	77.11	61.02	4.72*	70.18	61.00	76.02	22.97	29.38
Zn	(kg ha^{-1})	0.83	0.97	0.57^{ns}	0.706 a	1.302 a	0.690 b	0.58	27.22
	k (d^{-1})	0.009	0.015	8.53**	0.0092 b	0.0162 a	0.0106 ab	0.007	49.25
	$t_{1/2}$ (d)	94.97	66.14	4.50*	101.63	60.55	79.49	54.20	27.51
Mn	(kg ha^{-1})	2.87	2.53	0.23^{ns}	2.45	3.87	1.78	2.20	31.42
	k (d^{-1})	0.008	0.008	0.11^{ns}	0.008	0.011	0.006	0.006	28.28
	$t_{1/2}$ (d)	97.71	123.44	1.66^{ns}	108.54	87.07	136.11	61.84	49.40

Means followed by different letters in the same line differ according to the Tukey test to 5% level. ^{ns}not significant (P>0.05), *significant (P<0.05), **significant (P<0.01). SMD=Significant Mean difference; CV=Coefficient of variation.

In the second cover crop cycle there were significant differences between pruning times for the decomposition constant (k) of Mg, Cu, and Zn, and for the half-life of Mg and Cu. In the third cycle was found a significant difference only for the half-life of Cu. The lablab showed higher resistance to the release of this nutrient, with a half-life of 46.37 d. The half-life for Cu was also influenced by the grapevine pruning times, as the first time presented a long duration, 42.48 d. For K, the grapevine pruning time influenced the amount released with higher amounts in the first season (Table 6).

Table 6. Quantity released (kg ha^{-1}), release constant k (d^{-1}) and half-life ($t_{1/2}$) for nutrients of cover crops, lablab (LB), *C. ensiformis* (CE) and weeds (W), after 80 d in decomposition in the third consortium cycle and two grapevine pruning times.

Nutrient	Parameter	Pruning time		F	Treatments			SMD	CV (%)
		1st	2nd		LB	CE	W		
		3ndcycle							
N	(kg ha^{-1})	229.55	250.78	0.31^{ns}	248.08	248.00	224.41	117.20	43.11
	k (d^{-1})	0.049	0.049	0.005^{ns}	0.049	0.051	0.047	0.010	18.03
	$t_{1/2}$ (d)	14.49	14.68	0.04^{ns}	14.32	14.10	15.34	2.78	16.85
P	(kg ha^{-1})	27.80	25.51	0.32^{ns}	26.28	30.15	23.53	12.54	41.57
	k (d^{-1})	0.042	0.040	0.29^{ns}	0.040	0.044	0.039	0.011	23.30
	$t_{1/2}$ (d)	17.51	18.03	0.23^{ns}	18.20	16.44	18.68	4.69	23.31
K	(kg ha^{-1})	52.34	40.67	7.83*	46.94	45.37	47.20	12.93	24.57
	k (d^{-1})	0.043	0.038	2.27^{ns}	0.039	0.042	0.040	0.010	21.01
	$t_{1/2}$ (d)	16.80	18.85	3.25^{ns}	18.28	17.06	18.14	3.52	17.45
Ca	(kg ha^{-1})	83.24	74.32	0.45^{ns}	79.51	83.94	72.89	41.19	46.18
	k (d^{-1})	0.039	0.039	0.004^{ns}	0.038	0.041	0.037	0.012	26.94
	$t_{1/2}$ (d)	19.30	18.85	0.05^{ns}	19.74	17.36	20.12	6.15	28.49
Mg	(kg ha^{-1})	8.46	7.34	1.35^{ns}	8.45	8.02	7.23	2.96	33.09
	k (d^{-1})	0.042	0.041	0.03^{ns}	0.041	0.044	0.040	0.012	25.34
	$t_{1/2}$ (d)	17.56	17.61	0.001^{ns}	17.84	16.80	18.10	4.60	23.13
Cu	(kg ha^{-1})	0.22	0.30	2.11^{ns}	0.18	0.29	0.32	0.17	30.31
	k (d^{-1})	0.020	0.025	1.82^{ns}	0.016	0.026	0.026	0.010	38.70
	$t_{1/2}$ (d)	42.48	26.94	15.42**	46.37 a	28.33 b	29.44 b	12.26	31.19
Zn	(kg ha^{-1})	0.72	0.69	0.05^{ns}	0.69	0.72	0.71	0.34	42.03
	k (d^{-1})	0.061	0.061	0.08^{ns}	0.059	0.063	0.061	0.010	14.19
	$t_{1/2}$ (d)	11.46	11.49	0.002^{ns}	11.80	11.10	11.53	1.78	13.71
Mn	(kg ha^{-1})	4.20	3.11	3.02^{ns}	4.02	3.72	3.22	1.92	46.57
	k (d^{-1})	0.055	0.051	0.89^{ns}	0.055	0.051	0.053	0.014	23.28
	$t_{1/2}$ (d)	13.33	14.33	0.52^{ns}	13.30	14.46	13.74	4.29	27.44

Means followed by different letters in the same line differ according to the Tukey test to a 5% level. ^{ns}not significant (P>0.05), *significant (P<0.05), **significant (P<0.01). SMD=Significant Mean difference; CV=Coefficient of variation.

In the first and third cycles, there is a similar behavior in the nutrient release parameters where the plants do not differ significantly for most nutrients. These results can be explained by the fact that the plants of the second cycle grew longer until management (105 d), while in the first and third cycles the plants grew no more than 60 d. Therefore, the age of the plant can be associated with its nutrient composition (Souza *et al*., 2012). While the young plants are more tender and have a higher decomposition rate, older plants have most of their parts lignified and,

therefore, are more resistant to decomposition and present a consequent lower release of nutrients. Besides, the first and third cycles coincided with periods of the year with higher temperatures and higher rainfall, favoring the decomposition of the straw. For the second cycle, although there was irrigation, and the thermal amplitude was lower, which may decrease the decomposition activity of the straw.

Soil fertility

There was no significant difference among the cover crops for soil chemical attributes after three cultivation cycles of two grapevine crops (Table 7).

Comparing the values before and after the cover crop, the increase of P, K, Ca, and H+Al level is clear. According to Collier *et al*. (2011), this is due to possibly intake of these nutrients after decomposition of the previous straw. Silva *et al*. (2002) also observed Ca increase after the implementation of intercropped leguminous family species in an orange-pear orchard, compared to the ground situation before the experiment. Faria *et al*. (2004), using leguminous cover crops with vineyards under Ultisol in Petrolina (state of Pernambuco) after eight years, noted several improvements in the chemical characteristics of the soil, including an increase the exchangeable Ca in the 0-10 cm depth compared to the control without cover crops. Rosa *et al*. (2009) found that acidity and nutrient availability in the soil were influenced by cover crops associated with the grapevine, in the mountainous region of the state of Rio Grande do Sul.

Table 7. Chemical attributes of Red Latosol (Oxisol) before experiment installation (collection conducted March 8th, 2013) and after three cycles (collection on December 16th, 2013) with the cultivation of grapevine intercropped with cover crops.

Parameters	Initial level	Cover crop treatment			SMD	CV(%)
		Lablab	*C. ensiformis*	Weeds		
pH ($CaCl_2$)	6.20	6.11	6.03	6.12	0.18	2.68
P (Mehl) (mg dm^{-3})	3.80	8.83	9.33	5.03	7.23	40.33
K (mg dm^{-3})	105.00	124.70	129.40	111.00	34.26	24.87
Ca ($cmol_c\ dm^{-3}$)	5.60	6.27	6.57	6.55	0.98	13.41
Mg ($cmol_c\ dm^{-3}$)	2.60	1.79	1.73	1.69	0.54	27.54
H+Al ($cmol_c\ dm^{-3}$)	1.70	2.42	2.65	2.46	0.33	11.63
SB ($cmol_c\ dm^{-3}$)	8.47	8.35	8.31	8.90	1.32	13.72
CEC ($cmol_c\ dm^{-3}$)	10.17	10.93	10.75	11.41	1.43	11.51
O.M. (g dm^{-3})	38.00	17.50	18.30	13.00	7.27	39.50
V (%)	81.50	76.34	77.28	77.69	4.02	4.61

Means of treatments followed by different letters in the same line were different according to the Tukey test with a 5% of significance. H+Al= potencial activity; SB = sum of basic cations; CEC = cation exchange capacity; OM = organic matter; V = base saturation. SMD= significant mean difference, CV= coefficient of variation.

Nascimento *et al*. (2003) studied the effect of several tropical herbaceous legumes, cultivated as cover crops, on the chemical characteristics of a degraded Luvisol. According to their findings, it was observed significant effects of the legumes on soil fertility with significant increases in pH and exchangeable bases, positively reflecting on the CEC and base cation saturation index. Despite the cover crops promoting discrete soil acidification by raising H+Al levels and reducing organic matter levels, there was no increase in exchangeable Al that remained null in all soil samples.

The K content in soil increased 19.7 mg dm^{-3} in the plots cultivated with lablab, 24.4 mg dm^{-3} with *C. ensiformis* and 6 mg dm^{-3} with weeds. The P content in the soil also increased reporting values of 5.03 mg dm^{-3}, 5.53 mg dm^{-3}, and 1.23 mg dm^{-3} in the plots cultivated with lablab, *C. ensiformis*, and weeds, respectively. On the other hand,

Cardoso *et al*. (2013) found that the P content in the soil increased by 0.6 mg dm^{-3} when cultivated with *C. ensiformis* and millet. According to the authors, this P increase may be related to the ability of these plants to absorb the P subsurface soil layers and make it available on the surface, after the decomposition of straw. Such association can also be attributed to an element of easily leaching such as K, and plants with deeper roots can cycle this nutrient.

Negative effects were observed for Mg, soil organic matter, and basic cation saturation; showing decreasing levels in the soil at the end of the experiment. The organic matter content was reduced in the plots with weeds from 38 g dm^{-3} to 13 g dm^{-3}. For lablab, the reduction was lower, reflecting the effect of higher biomass production by these plants, thereby maintaining a good level of organic matter in the soil. Collier *et al*. (2011) found, in treatment with *C. ensiformis*, decrease of organic matter in soil because of a positive priming effect, to stimulate the soil biota in the decomposition of the existing organic matter. The activating effect (priming) is defined as the rapid change of the organic carbon and nitrogen content of the soil. It can be positive (mineralization of C and N) by adding low C/N ratio materials or nitrogen mineral fertilizers. Otherwise, this effect can be negative (net immobilization) by the addition of high C/N material (Buso and Kliemann, 2003).

Nutritional status of grapevine

Cover crops did not affect the nutritional status of the grapevines at blooming in both crop seasons (Table 8). A different outcome was noticed by Zalamena *et al*. (2013), who verified lower content of P and K in the leave of grapevines planted with species of cover crops compared to the control treatment (weeds). This reduction can be attributed to the higher absorption and accumulation of both elements in the tissue of cover crops, reducing the availability in the soil for grapevine plants (Celette *et al*., 2009; Brunetto *et al*., 2011).

According to Celette *et al*. (2009), along with the grapevine cycle, the cover crop plants also absorb the water and nutrients from the soil solution, especially N, which may even reduce the availability of this element to the grapevine. Thus, increase in total N content in the leave of grapevines intercropped with annual cover crops is not normally expected, and may even be the opposite, as Wheeler *et al*. (2005) noted.

N, P and K levels were higher in the summer season compared to winter. This increase can be attributed to the fact that grapevine plants may have benefited from the nutrients released by the decomposition of previous cover crops and soil organic matter at the second harvest since there had already been two cycles of cover crops. It can be inferred that the use of cover crops over time provides better nutrient availability in the soil, with consequent benefits for the main crop. Faria *et al*. (2004) studied changes in soil characteristics after eleven legume cycles with nine grapevine crops seasons and noticed a soil fertility improvement in the sixth and ninth seasons.

For grapevine, and most of the crops, the standard levels of nutrients that are correlated with the higher production are not well established, but it is possible to work with a concentration range for the interpretation of the results. The concentration ranges recommended by the Agronomic Institute of Campinas for grapevine plants are divided into five levels: deficiency, slight deficiency, normal, slight excess and excess (Table 2) (Terra and Tecchio, 2008).

The grapevine plants showed a slight N deficiency in the winter season, however, in the summer season, the N content was in the optimal (normal) range, reinforcing the use of this nutrient arising from the decomposition of cover crops and soil organic matter. The plant P content in the winter and summer season showed a slight excess. According to Mafra *et al*. (2011), the grapevine has a low demand for P, that is attributed to the association of grapevines with mycorrhizal fungi present in the roots of plants in poor soils, which exploit little soluble forms of this element. However, this is not the case of the present work, because the P content in the soil presented as low to medium, according to Sousa *et al*. (2004) (Table 6), so if there was a mycorrhizal association, it might have contributed to increasing the absorption of this nutrient.

The K content in the grapevines, for the two crops, was framed within the slight deficiency range. Grapevine leave showed excess of Ca in the two seasons, except for treatment with the weeds in the winter crop, which was in

slight excess range. The scarcity level was observed for Mg in the two seasons, regardless of cover crops. The dynamics of these three nutrients in vineyards are very important, and the relationship between the nutrients, such as K/Mg and K/(Ca+Mg) should be considered. When there is an inverse relationship between these elements, especially high content of K and low Mg and Ca, an abiotic anomaly known as "desiccation of the rachis" can occur (Fráguas *et al*., 1996; Miele *et al*., 2009). According to Silva *et al*. (2005), high K, high Ca and low Mg levels also contributed to the emergence of desiccation of the rachis.

Table 8. Nutrients in grapevine canopy intercropped with, lablab (LB), *C. ensiformis* (CE) and weeds (W) and grapevine pruning times.

Nutrient	Harvest	Pruning times		F	Treatments			SMD	CV(%)
		1st	2nd		LB	CE	W		
N (g kg^{-1})	Winter	27.61	26.65	1.19 ^{ns}	26.80	26.69	27.91	2.72	8.88
	Summer	35.44	33.00	7.64 *	34.61	34.28	33.77	2.73	7.06
P (g kg^{-1})	Winter	4.44	2.63	99.03 **	3.55	3.54	3.52	0.56	14.10
	Summer	4.43	4.28	1.04 ^{ns}	4.53	4.29	4.24	0.44	9.06
K (g kg^{-1})	Winter	8.13	7.97	0.09 ^{ns}	8.18	7.54	8.44	1.61	17.74
	Summer	11.68	11.70	0.01 ^{ns}	11.62	11.68	11.78	0.70	5.32
Ca (g kg^{-1})	Winter	25.40	44.86	13.92 **	35.80	33.50	21.10	24.48	35.25
	Summer	32.48	31.78	0.07 ^{ns}	32.24	31.94	32.22	8.30	22.82
Mg (g kg^{-1})	Winter	2.00	3.07	17.18 **	2.80	2.30	2.50	0.79	27.82
	Summer	2.63	2.62	0.008 ^{ns}	2.60	2.67	2.61	0.60	20.21
Cu (mg kg^{-1})	Winter	11.26	10.33	0.50 ^{ns}	10.80	10.20	11.40	4.07	33.30
	Summer	11.27	12.00	2.78 ^{ns}	12.20	11.30	11.40	1.36	10.35
Fe (mg kg^{-1})	Winter	366.53	424.07	24.71 **	402.30	384.60	399.00	35.87	8.02
	Summer	179.40	181.13	0.008 ^{ns}	164.30	191.20	185.30	59.41	29.11
Mn (mg kg^{-1})	Winter	170.33	182.66	0.12 ^{ns}	179.60	162.10	187.80	111.29	55.70
	Summer	92.00	113.93	7.06 *	93.60	104.10	111.20	25.58	21.95
Zn (mg kg^{-1})	Winter	26.01	28.28	0.770 ^{ns}	26.61	26.71	28.12	8.02	26.10
	Summer	21.93	21.80	0.003 ^{ns}	21.10	19.60	24.90	7.58	30.65

Means of treatments followed by different letters in the same line were different according to the Tukey test with a 5% of significance. SMD= significant mean difference, CV= coefficient of variation.

For micronutrients in cover crop treatments, the Cu was framed as under slight deficiency in two crops seasons. The Fe content was in the excess range in the winter season and a slight excess in the summer season. The Mn content fell in the slight excess range in two crop seasons. A slight deficiency was observed for Zn content in grapevine leave, in two crops seasons.

The nutrient release parameters did not differ among the evaluated cover crops but depend on the grapevine pruning time. Cover crops did not change the soil chemical attributes of soil fertility in three crop cycles and two grapevine crops. Cover crops did not affect the nutritional status of the grapevines at blooming in the two evaluated times.

CONCLUSIONS

Canavalia ensiformis is more efficient in the accumulation of nutrients in its aerial parts than other cover crops.

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REFERENCES

Albarello JB, Magro R, Melo GW, Freitas RF, Rodighero K and Oliveira PD. 2013. Efeito da adição da parte aérea de plantas de cobertura na fitotoxidade de cobre em solo cultivado com videira. pp. 1-4. In: XXXIV Congresso Brasileiro de Ciêcia do Solo. Brazilian Soil Science Society. Florianapolis

Bataglia OC, Furlani AMC, Teixeira JPF, Furlani PR and Gallo JR. 1983. Métodos de análise química de plantas. Technical Bulletin Nº. 78. Agronomic Institute of Campinas. 48 p.

Bertin EG, Andrioli I and Centurion JF. 2005. Plantas de cobertura em pré-safra ao milho em plantio directo Acta Scientiarum Agronomy 27(3): 379-386. doi: 10.4025/actasciagron.v27i3.1393

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JA, Cunha TJF and Oliveira JB. 2013. Sistema brasileiro de classificação de solos. Third Edition. Embrapa, Brasília. 353 p.

Brazilian Agricultural Research Corporation -Embrapa. 1997. Manual de Métodos de Análise de Solo. Second Edition. Embrapa-CNPS, Rio de Janeiro. 212 p.

Brunetto G, Ventura M, Scandellari F, Ceretta CA, Kaminski J, Melo G and Tagliavini M. 2011. Nutrient release during the decomposition of mowed perennial ryegrass and white clover and its contribution to nitrogen nutrition of grapevine. Nutrient Cycling in Agroecosystems 90(3): 299-308. doi: 10.1007/s10705-011-9430-8

Buso WHD and Kliemann HJ. 2003. Relações de Carbono Orgânico e de Nitrogênio Total e Potencialmente Mineralizável Com o Nitrogênio Absorvido pelo Milheto. Pesquisa Agropecuária Tropical 33(2): 97-105.

Calonego JC, Gil FC, Rocco VR and Santos EA. 2012. Persistência e Liberação de Nutrientes da Palha de Milho, Braquiária e Labe-Labe. Bioscience Journal 28(5): 770-781.

Cardoso DP, Silva MLN, de Carvalho GF, de Freitas DAF and Junior Avanzi JC. 2013. Espécies de plantas de coberturano recondicionamento químico e físico do solo. Revista Brasileira de Ciências Agrárias 8(3): 375-382. doi: 10.5039/agraria.v8i3a2421

Cavalcante VS, Santos VR, Santos Neto AL, dos Santos MAL, dos Santos CG and Costa LC. 2012. Biomassa e extração de nutrientes por plantas de cobertura. Revista Brasileira de Engenharia Agrícola e Ambiental 16(5): 521-528. doi: 10.1590/S1415-43662012000500008

Celette F, Findeling A and Gary C. 2009. Competition for nitrogen in an unfertilized intercropping system: The case of an association of grapevine and grass cover in a Mediterranean climate. European Journal of Agronomy 30(1): 41-51. doi: 10.1016/j.eja.2008.07.003

Collier LS, Kikuchi FY, Benício LPF and Sousa SA. 2011. Consórcio e sucessão de milho e feijão-de-porco como alternativa de cultivo sob plantio direto. Pesquisa Agropecuária Tropical 41(3): 306-313. doi: 10.5216/pat.v41i3.8706

Crusciol CAC, Moro E, Lima EV and Andreotti M. 2008. Taxas de decomposição e de liberação de macronutrientes da palhada de aveia preta em plantio direto. Bragantia 67(2): 261-266. doi: 10.1590/S0006-87052008000200024

De-Polli H and Chada SS. 1989. Adubação verde incorporada ou em cobertura na produção de milho em solo de baixo potencial de produtividade. Revista Brasileira de Ciência do Solo 13(3): 287-293.

Duarte Junior JB and Coelho FC. 2008. Adubos verdes e seus efeitos no rendimento da cana-de-açúcar em sistema de plantio direto. Bragantia 67(3): 723-732. doi: 10.1590/S0006-87052008000300022

Eissenstat DM. 2007. Dinamica di crescita delle radici nelle colture da frutto. Italus Hortus 14(1): 1-8.

Faria CMB, Soares JM and Leão PCS. 2004. Adubação verde com leguminosas em videira no submédio São Francisco. Revista Brasileira de Ciência do Solo 28(4): 641-648. doi: 10.1590/S0100-06832004000400006

Ferreira LE, Souza EP and Chaves AF. 2012. Adubação Verde e Seu Efeito Sobre os Atributos do Solo. Revista Verde 7(1): 33-38.

Fourie JC. 2012. Soil management in the Breed River Valley wine grape region, South Africa. 4. Organic matter and macro-nutrient content of a medium-textured Soil. South African Journal of Enology and Viticulture 33(1): 105-114. doi: 10.21548/33-1-1312

Fráguas JC, Sônego OR and Grigoletti A. 1996. O dessecamento do cacho de uva. Technical Bulletin Nº. 19. EMBRAPA-CNPUV. 4 p.

Gama-Rodrigues AC, da Gama-Rodrigues EF and de Brito EC. 2007. Decomposição e liberação de nutrientes de resíduos culturais de plantas de cobertura em argissolo vermelho-amarelo na região noroeste Fluminense (RJ) Revista Brasileira de Ciência do Solo 31(6): 1421-1428. doi: 10.1590/S0100-06832007000600019

Giongo V, Mendes AMS, Cunha TJF and Galvão SRS. 2011. Decomposição e liberação de nutrientes de coquetéis vegetais para utilização no Semiárido brasileiro. Revista Ciência Agronômica 42(3): 611-618. doi: 10.1590/S1806-66902011000300006

Guerra JGM and Teixeira MG. 1997. Avaliação inicial de algumas leguminosas herbáceas perenes para utilização como cobertura viva permanente de solo. Technical Bulletin No 16. EMBRAPA-CNPAB. 7 p.

Instituto Nacional de Meteriologia-INMET. 2014. Estação Meteorológica de Observação de Superfície Automática. In: Estações Automáticas, http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas.

Leite LF, Freitas RDC, Sagrilo E and Galvão SR. 2010. Decomposição e liberação de nutrientes de resíduos vegetais depositados sobre Latossolo Amarelo no Cerrado Maranhense. Revista Ciência Agronômica 41(1): 29-35. doi: 10.5935/1806-6690.20100004

Mafra MSH, Cassol PC, Miquelluti DJ, Ernani PR, Gatiboni LC, Ferreira EZ, Barros M, Zalamena J and Grohskopf MA. 2011. Atributos químicos do solo e estado nutricional de videira Cabernet Sauvignon (Vitis vinifera L.) na Serra Catarinense. Revista de Ciências Agroveterinárias 10(1): 44-53.

Miele A, Rizzon LA and Giovanini E. 2009. efeito do porta-enxerto no teor de nutrientes em tecidos da videira 'cabernet sauvignon'. Revista Brasileira de Fruticultura 31(4): 1141-1149. doi: 10.1590/S0100-29452009000400031

Nachtigal JC and Schneider EP. 2007. Recomendações para produção de videiras em sistemas de base ecológica. Document 65. Embrapa uva e vinhos, Bento Gonçalves. 70 p.

Nascimento JT, da Silva IF, Santiago RD and da Silva Neto LF. 2003. Efeito de leguminosas nas características químicas e matéria orgânica de um solo degradado. Revista Brasileira de Engenharia Agrícola e Ambiental 7(3): 457-462. doi: 10.1590/S1415-43662003000300008

Padovan MP, Motta IS, Carneiro LF, Moitinho MR and Fernandes SSL. 2011. Dinâmica de acúmulo de massa e nutrientes pelo feijão-

de-porco para fins de adubação verde e o estádio mais adequado para manejo. Revista Brasileira de Agroecología 6(3): 182-190.

Paul EA and Clark FE. 2007. Soil microbiology, ecology, and biochemistry. Third edition. Academic Press, San Diego. 273 p.

Rosa JD, Mafra AL, Nohatto MA, Ferreira EZ, Oliveira OLP, Miquelluti DJ, Cassol PC and Medeiros JC. 2009. Atributos químicos do solo e produtividade de videiras alterados pelo manejo de coberturas verdes na Serra Gaúcha. Revista Brasileira de Ciência do Solo 33(1): 179-187. doi: 10.1590/S0100-06832009000100019

Saminêz TCO, Resende FV, Souza AF and Carvalho AM. 2006. Extraction of nutrients by green manure species under organic production system in the Cerrado summer conditions. Revista Brasileira de Agroecología 1(1): 1011-1014.

Silva DJ and Leão PCS. 2005. Dessecamento da ráquis em uva Thompson Seedless cultivada no Submédio São Francisco. In: Latin American Congress of viticulture and enology. Bento Gonçalves.

Silva JAA, Vitti GC, Stuchi ES and Sempionato OR. 2002. Reciclagem e Incorporação de Nutrientes ao Solo Pelo Cultivo Intercalar de Adubos Verdes em Pomar de Laranjeira-'Pêra'. Revista Brasileira Fruticultura 24(1): 225-230. doi: 10.1590/S0100-29452002000100048

Soil Survey Staff. 1999. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. Second Edition. Unites States Department of Agriculture (USDA), Washington, DC. 884 p.

Sousa DMG, Lobato E and Rein TA. 2004. Chapter 6: Adubação com fósforo . pp. 147-167. In: Sousa DMG and Lobato E (ed.). Cerrado: correção do solo e adubação. Second Edition. Embrapa Tenological information, Brasília. 416 p.

Souza CM, Pires FR, Partelli FL and Assis RL. 2012. Adubação verde e rotação de culturas. First Edition. UFV, Viçosa. 108 p.

Suzuki LEAS and Alves MC. 2006. Fitomassa de Plantas de Cobertura em Diferentes Sucessões de Culturas e Sistemas de Cultivo. Bragantia 65(1): 121-127. doi: 10.1590/S0006-87052006000100016

Teixeira LAJ, Tecchio MA, Moura MF, Terra MM, Pires EJP and Hernandes JL. 2011. Alterações em Atributos Químicos de um Solo Submetido à Adubação e Cultivado com Videira 'Niagara Rosada'. Revista Brasileira de Fruticultura 33(3): 983-992. doi: 10.1590/S0100-29452011000300035

Terra MM and Tecchio MA. 2008. Nutrição e Adubação para Uvas Rústicas. pp. 159-173. In: Boliani AC, Fracaro AA and Corrêa LS (ed.). Uvas rústicas: cultivo e processamento em regiões tropicais. University Graphics & Publishing. Jales. 368 p.

Thomas RJ and Asakawa NM. 1993. Decomposition of leaf litter from tropical forage grasses and legumes. Soil Biology and Biochemistry 25(10): 1351-1361. doi: 10.1016/0038-0717(93)90050-L

Wheeler SJ, Black AS and Pickering GJ. 2005. Vineyard floor management improves wine quality in highly vigorous *Vitis vinifera* 'Cabernet Sauvignon' in New Zealand. New Zealand Journal of Crop and Horticultural Science 33(3): 317-328. doi: 10.1080/01140671.2005.9514365

Zalamena J, Cassol PC, Brunetto G, Grohskopf MA and Mafra MSH. 2013. Estado nutricional, vigor e produção em videiras cultivadas com plantas de cobertura. Revista Brasileira de Fruticultura 35(4): 1190-1200. doi: 10.1590/S0100-29452013000400030

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Identification of climatic and physiological variables associated with rice (*Oryza sativa* L.) yield under tropical conditions

Identificación de variables climáticas y fisiológicas asociadas al rendimiento del arroz (*Oryza sativa* L.) en condiciones tropicales

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ABSTRACT

Rice crop productivity is influenced by climatic conditions such as solar radiation, temperature, and water availability during its vegetative and reproductive stage. In Colombia, rice cultivation is carried out throughout the year; so, it is necessary to identify the sowing dates where high yields are obtained, and which physiologic and climatic factors significantly influence them. Therefore, this research aimed to identify the key climatic and physiological factors that allow maximizing the yield and maintaining good productivity in sowing dates with optimal and deficient environmental conditions, respectively. The experiment was carried out in a rice producing region in northern of Tolima, Colombia from 2015 to 2016. Ten sowing dates were established, with a randomized complete block design in a divided strips arrangement. For each sowing date, climatic conditions were tracked, and growth, development, and yield of rice plant were evaluated. Also, the photosynthetic rate was assessed on five sowing dates. Results showed that physiologic factors that have more relation with crop yield are plant height, leaf area index and dry mass accumulation between phenological stages 37 and 49; whereas the unique climatic factor, that was highly related to yield, was solar radiation between phenological stages 51 to 77. Furthermore, when the optimum values of each variable were reached, a yield higher than 9,500 kg ha^{-1} was achieved. No relation was observed between the photosynthesis rate of at leaf level and yield.

Keywords:
Crop yield
cv. *Oryzica* 1
Photosynthesis
Solar radiation
Sowing date

RESUMEN

La productividad del cultivo del arroz está influenciada por las condiciones climáticas, como la radiación solar, temperatura y disponibilidad de agua, durante la etapa vegetativa y reproductiva. En Colombia se realizan siembras de arroz durante todo el año, por lo que es necesario identificar las fechas de siembra donde se obtenga alto rendimiento, y qué factores fisiológicos y climáticos influyen de forma significativa en este. Por lo tanto, esta investigación tuvo como objetivo identificar los factores climáticos y fisiológicos clave, que permitan maximizar el rendimiento y mantener una buena productividad en fechas de siembra con condiciones ambiental óptimas y deficientes, respectivamente. El experimento se realizó en una región productora de arroz en el norte de Tolima, Colombia durante los años 2015 y 2016. Se establecieron diez fechas de siembra, con un diseño en bloques completos al azar en un arreglo de franjas divididas. En cada fecha de siembra se hizo seguimiento a las condiciones climáticas y se evaluó el crecimiento, desarrollo y rendimiento de las plantas de arroz. Además, la tasa fotosintética se evaluó en cuatro fechas de siembra. Se encontró que los factores fisiológicos que más relación tienen con el rendimiento son la altura de la planta, el índice de área foliar y la acumulación de masa seca entre los estados fenológicos 37 y 49, mientras que, un único factor abiótico que estuvo altamente relacionado con el rendimiento fue la radiación solar entre los estados fenológicos 51 a 77. Cuando se alcanzaron los valores óptimos de cada una de estas variables se alcanza un rendimiento superior a los 9.500 kg ha^{-1}. No se observó relación entre la tasa de fotosíntesis a nivel de hoja y el rendimiento.

Palabras clave:
Rendimiento de cultivos
cv. *Oryzica* 1
Fotosíntesis
Radiación solar
Fecha de siembra

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Rice (*Oryza sativa* L.) is an essential food grain for about half of humanity, being a basic component in political, economic and social stability, and to a certain degree, in our survival (Degiovanni *et al*., 2010). Rice is the second most cultivated cereal in the world after corn reaching a world production of 740 billion t in 2014; in Colombia production reached 2.2 million t the same year (FAO, 2017).

Climatic conditions directly affect crop physiology and yield (Chen *et al*., 2004; Jarma *et al*., 2012). Currently, climate change has generated climatic alterations as a higher frequency of extreme weather events (Delerce *et al*., 2016). Climate change effects on different zones vary depending on the magnitude and seasonal characteristics (Ko *et al*., 2014). In Colombia, according to the IPCC (2014), these events will occur in higher frequency and magnitude, which, according to simulation models, will generate a temperature increase from 5 to 7 °C and an approximate decrease of 10% in precipitation during 2005-2100 period. In this context, there will be variability in rice cultivation productivity between 5 and 29% (Iizumi *et al*., 2014), which represents a threat at the socioeconomic level for rice producers (Delerce *et al*., 2016).

Rice productivity is influenced by climatic conditions such as Solar Radiation (SR), temperature and water availability during the vegetative and reproductive stages (Fageria, 2007). For example, high night temperature (>30 °C) reduces crop yield; it causes an increase in respiratory rate, subsequently, reduces photosynthesis rate, amount of dry matter (DM), and leaf area (Alvarado *et al*., 2017). Another climatic variable that negatively affects rice cultivation is high daytime temperature (>40 °C), as this generates an increase in respiratory rate, and therefore, a reduction in photosynthesis rate by non-stomatal limitations (Sánchez *et al*., 2014). DM production and harvest index are positively related to yield (Yoshida, 1981). Accumulation of DM is determined by SR interception by the canopy, which is influenced by the amount of incident SR and characteristics such as Leaf Area Index (LAI) and insertion angle and orientation (Ying *et al*., 1998; De Costa *et al*., 2006; Zhang *et al*., 2009). The LAI has a direct correlation with yield because it determines the ability to intercept a more considerable amount of photosynthetically active radiation (Ahmad *et al*., 2009; Aschonitis *et al*., 2014). Therefore, SR is the limiting factor of productivity (Delerce *et al*., 2016).

In the department of Tolima, Colombia, agronomic management practices as sowing dates (SD) are not carried out in accordance with the region's climatology. Producers are sowing throughout the year due to water access rotation imposed by irrigation districts, so the two SD with the highest solar radiation peaks are being misapplied. Thus, there is a high yield variability in different SD (Castilla *et al*., 2010; Delerce *et al*., 2016). Nonetheless, because of climate change, the SD that is considered ideal for crop establishment may have suffered changes.

Availability of climatic information and plant characteristics help understand yield variability and its determinant factors (Huang *et al*., 2016). Consequently, for this production area, it is necessary to estimate the physiologic and climatic factors that are highly related to yield, with the objective of using them as variables for crop monitoring or defining management practices to maintain these key variables within optimal ranges.

In the department of Tolima, the effect of three SD on crop growth and yield have been studied (Garcés and Restrepo, 2015). Besides, some authors have carried out studies with secondary information to estimate the relationship between climatic variables and yield (Delerce *et al*., 2016). Previous investigations were developed in the municipality of Saldaña in the south of the department, but this production area is very different in climate, soil characteristics, and management practices applied to productive rice systems compared to the north of Tolima. There is a necessity in development studies that evaluate how the physiological and climatic factors affect rice crop in this region. Therefore, this research aimed to identify the key climatic and physiological factors that allow maximizing the yield and maintaining good productivity in sowing dates with optimal and deficient environmental conditions, respectively. In the experiment, 10 SD were evaluated to identify which time of the year presented the best environmental conditions that allow maximizing the crop yield and establishing a relationship through automatic learning techniques between physiologic and climatic factors and the yield.

MATERIALS AND METHODS

Study site and plant material

This research was carried out during the period 2015-2016 in the municipality of Armero-Guayabal, located in the northern region of Tolima, Colombia. Ten sowing dates (SD), a SD per month, were established on November, December, January, February, March, April, May, June, July, and August. The cultivar used was *Oryzica* 1, this cultivar was selected because it has been sown for 34 years in this region due to its good productivity and high grain quality.

Experimental design and evaluated variables

The experiment was carried out in soil formed by volcanic flows with a sandy loam texture. Plots were established in a completely randomized block design in a divided strips arrangement, where the stripes corresponded to the sowing dates. Each strip comprised 2500 m^2 divided into three blocks. Similar agronomic management was carried out for the ten sowing dates, where irrigation was provided by gravity with a frequency of two days per week. The control of pests, diseases, and weeds were carried out according to weekly sampling, using chemical synthesis products. The nutritional supply was made using five edaphic fertilizations, during the different phenological stages according to the nutritional requirements for the extraction of potential yield of 10,000 kg ha^{-1}.

Growth variables and trends were estimated, as follows: Leaf area index (LAI) and dry matter (DM) above the soil was determined following the methodology described by Degiovanni *et al.* (2010) and Garcés and Restrepo (2015), respectively. Phenology stages were characterized according to the BBCH scale (Lancashire *et al.*, 1991), and plant height was also measured. These variables were evaluated in all the plants that were in an area of 625 cm^2, from the phenological stage 11 to 99. Once the phenological stage 99 was reached, harvest index and plant components were estimated. Moreover, with data obtained from the panicle number per square meter, weight of 1,000 grains, the percentage and weight of filled grains, green paddy yield in kg ha^{-1} was calculated according to Garcés and Restrepo (2015). The yield was estimated with a grain humidity of 22%.

Climatological variables were collected using a weather station (Davis Vantage Pro 2, San Francisco, California) located 500 meters far from the study area. These data were divided into 12 parts according to the BBCH scale, and the following variables were obtained: maximum diurnal temperature, maximum night temperature, minimum night temperature, accumulated solar radiation (SR), accumulated precipitation and average relative humidity according to each of the 12 parts considered.

In the SD of April, May, June, July, and August, photosynthesis rate, stomatal conductance and transpiration, in the phenological stages 49 (i.e., first awns visible), 65 (i.e., 50% of flowers), and 75 (i.e., milky ripe) were estimated in the youngest completely expanded leaf, using a portable open photosynthesis-meter system (LI-6400 XT Li-Cor Lincoln, Nebraska, U.S.A.). The photosynthetic photon flux density of 1600 µmol photons m^{-2} s^{-1}, the concentration of CO_2 inside the chamber was set at 400 µmol CO_2 mol^{-1}, and the vapor pressure deficit remained between 1.5 and 1.7 kPa; data were taken after reaching stable state equilibrium (~10 min). The area of the leaf inside the chamber was measured in order to correct with the real data area.

Growth trends, as well as growth, development, yield components and, climatic variables, were analyzed with the automatic learning methodology using tree decision algorithms (Delerce *et al.*, 2016). In order to use this technique, it is necessary to previously filter the variables considering their correlation with predictors (Hastie *et al.*, 2009); therefore, these variables were filtered by partial least squares regression using the NIPALS model (Geladi and Kowalski, 1986). Furthermore, the criteria considered were the variables of importance that presented a coefficient >0.8 and a correlation with the predictor variable. Once important variables were identified through the trees and photosynthesis analysis, contour plots were made with yield as a response variable. This analysis was done with the objective of identifying optimal points where performance is maximum (Figueroa, 2003). Besides, a multivariate analysis of variance with a Hotelling's significance test was performed with yield component data. Data analyzes were carried out with the software RStudio Inc., version 3.5.1.

RESULTS AND DISCUSSION

Identification of sowing dates

The SD of December and May presented the highest yields (Table 1), agreeing with the results found by various authors (Datt *et al.*, 2012; Sameera *et al.*, 2016; Dong

et al., 2017). Moreover, the number of panicles and the percentage of full grains have a high correlation with yield (Table 2) where a significant correlation is observed between these variables. This correlation explains the high yields found in the SD of December and May, the results obtained were similar to those reported by Thippani *et al*. (2017). However, no relationship was observed between the weight of 1,000 grains and the number of grains per panicle with yield (Table 2), what differs from what was found by Díaz *et al*. (2000), where they stated that these are variables that significantly influence yield and are closely related to grain length. Possibly this correlation was not observed because only one variety was evaluated, and it is probable that no variability in this character would occur, so in future studies, this variable should be considered.

Table 1. Yield components and harvest index of rice in ten sowing dates in Armero-Guayabal.

Sowing date	Harvest index	Number of panicles (m^2)	Yield (kg ha^{-1})	Weight of 1000 grain (g)	Filled grains (%)	Number grains per panicles	Hotelling grouping
July	0.42	260.00	6,247.46	24.53	65.14	85.59	a
May	0.37	509.67	9,964.31	21.74	77.31	78.60	b
December	0.30	569.67	10,831.3	24.70	78.62	59.23	c
November	0.43	347.17	5,658.62	24.85	78.69	50.74	d
April	0.38	359.17	9,609.48	20.93	79.54	62.83	e
March	0.28	353.33	7,992.42	24.63	71.92	76.78	fg
August	0.36	298.33	6,948.08	23.75	59.92	85.98	fhi
June	0.37	336.00	8,150.35	25.08	73.31	66.08	g
February	0.49	337.00	7,223.23	25.50	64.78	76.71	h
January	0.42	432.33	6,355.61	23.85	65.87	51.50	i

Treatments with a different letter indicate significant differences ($P \leq 0.05$).

Table 2. Pearson correlation between crop yield and yield components evaluated on ten sowing dates in Armero-Guayabal.

Response Variable 1	Response Variable 2	Pearson	*P*-value
Yield	Number of panicles	0.57687	0.0001
	Weight of 1,000 grains	-0.17424	0.28225
	Filled grains (%)	0.59497	0.00005
	Number of grain per panicle	0.19518	0.22746

Identification of physiologic and climatic factors associated with rice yield

According to the tree method, the LAI variable in the stage 49 (i.e. maximum panicle swelling stage), plant height in the phenological stage 41 (i.e. beginning of panicle swelling), and accumulated DM in the stage 37 (i.e. elongation of the stem), are the variables that are mostly associated with rice yield, moreover, together they explain this with an R^2 of 0.92 (Figure 1A). Maximum yield is reached when the LAI is between 10 and 11 (Figure 2A), which are similar to values found by Mae *et al*. (2006), who observed a linear relationship between LAI and yield, finding the maximum crop yield with a LAI between 10 and 12. Garcés and Restrepo

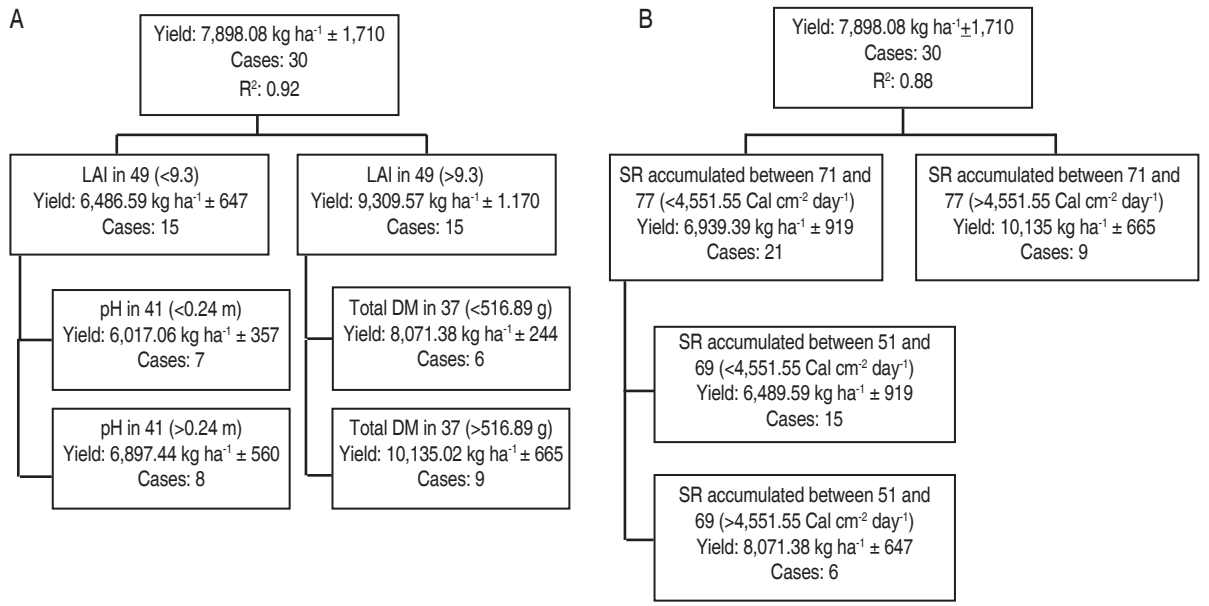

Figure 1. A. Tree for physiologic factors associated with rice yield; B. Tree for climatic factors associated with rice yield. LAI: leaf area index; PH: plant height; DM: dry matter; SR: solar radiation.

(2015) conducted a similar study in the southern area of Tolima, where they found that maximum yield was reached with a LAI of 7; this difference can be attributed to the fact that different varieties were used. The highest yield is reached with a plant height between 0.30 and 0.40 m, while yield is significantly reduced when it is less than 0.30 m (Figure 2A). These differences are present because higher plants allow better ventilation and better location of the leaves inside the canopy. On the contrary, plants with lower height generate lower ventilation and wrong leaf location, which generate a reduction in the photosynthesis rate of the canopy in 60 to 80%, and yield is reduced by approximately 2,000 kg ha^{-1} (Setter, 1997; Peng *et al.*, 2008). Therefore, to obtain a high yield, it is necessary to reach a LAI between 10 and 11 and a plant height between 0.30 and 0.40 m, both between plant development stages 41 and 49, which allows the plant to have a better solar radiation uptake.

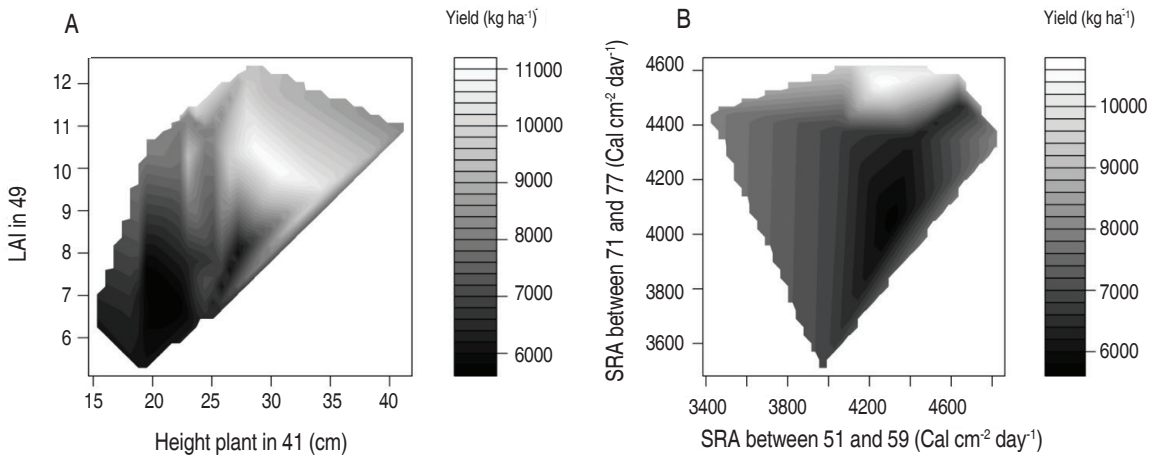

Figure 2. A. Physiologic contour plot associated with rice yield; B. Climatic factor contour plot associated with rice yield; LAI: leaf area index; SR: solar radiation.

SR that is received by crops from stages 30 to 89 has a decisive influence on crop yield (Yoshida and Parao, 1976). According to the tree methodology, the climatic factors that are the most associated with yield is SR in the phenological stage 51 to the 77, with an R^2 of 0.88 (Figure 1B). Maximum yield is reached when SR presented values between 4,000 and 4,500 cal cm^{-2} d^{-1} between stages 51 to 59 (i.e., inflorescence emergence), and greater than 4,500 cal cm^{-2} d^{-1} between stages 71 and 77 (i.e., development of fruit) (Figure 2B). The relationship found between SR and yield is due to a direct relationship between global and intercepted SR and accumulation of DM; and therefore, with yield (Garcés and Restrepo, 2015; Huang *et al*., 2016).

However, for SR to be converted into DM, it must be intercepted, and this is defined by plant LAI and architecture (Ying *et al*., 1998; De Costa *et al*., 2006; Zhang *et al*., 2009). These physiological factors are decisive because it allows SR, that cannot be absorbed by the leaves of the upper third, to penetrate and be intercepted by the middle and lower third leaves, where 70% of the leaf area is found, which contributes to a photosynthetic rate of approximately ~47% of the canopy (Song *et al*., 2013). Furthermore, to have a high efficiency in the conversion of SR into carbon skeletons, it is necessary that the photosynthetic apparatus does not show climatic stress limitations, which can be generated by high diurnal (>40 °C) and nocturnal (>30 °C) temperatures (Sánchez *et al*., 2014; Alvarado *et al*., 2017). However, these temperatures were not found in any sowing date (data not shown).

The photosynthesis rate has a close relationship with yield because as it increases, photosynthate supply increases from leaves to grains (Fu and Lee, 2008). However, no clear relationship was found between yield and this variable. SD with the highest yield does not coincide with a high photosynthesis rate in any of the three phenological stages evaluated (Figure 3). This lack of relationship is contradictory to the direct relationship between photosynthesis rate and yield found by other authors (Hidayati *et al*., 2016). This result is explained because the photosynthesis rate was not evaluated at the canopy level but on a single leaf, and it was also estimated at the saturation point and not the real photosynthesis rate on different phenological moments. Therefore, the influence of SD in the photosynthesis rate of the canopy should be studied further in depth, through daily curves, which allow seeing the real capacity of carbon fixation and its relationship with the yield.

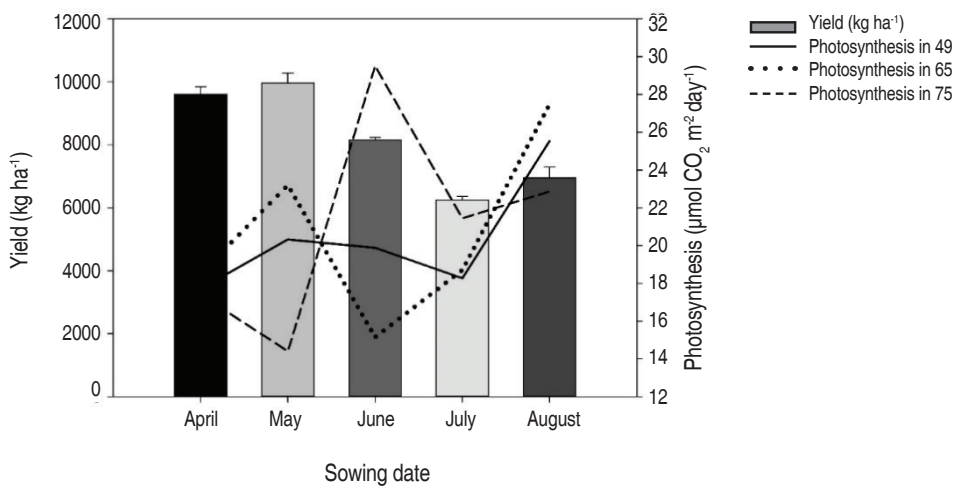

Figure 3. Behavior of yield and the photosynthetic rate in different phenological stages of the rice plant.

CONCLUSIONS

The physiological parameters that influenced the rice yield are leaf area index, plant height and dry matter in rice phenological stages 37 to 49. The climatic factor that had a significant relationship with yield was solar radiation in plant phenological stages between 51 and 77, corresponding to the sowing days of December and May. Since solar radiation was optimal for only these

sowing dates, more practices and studies should be developed to maximize solar radiation uptake along the year, focusing on the increase of photosynthesis rate of the canopy, and subsequently, rice yield.

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REFERENCES

Ahmad A, Iqbal S, Ahmad S, Khaliq T, Nasim W, Husnain Z, Hussain A, Zia-ul-Haq M and Hoogenboom G. 2009. Seasonal growth, radiation interception, its conversion efficiency and biomass production of *Oryza sativa* L. under diverse agro-environments in Pakistan. Pakistan Journal of Botany 41(3): 1241–1257.

Alvarado O, Garces G and Restrepo H. 2017. The effects of night-time temperatures on physiological and biochemical traits in rice. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 45(1): 157–163. doi: 10.15835/nbha45110627

Aschonitis VG, Papamichail DM, Lithourgidis A and Fano EA. 2014. Estimation of leaf area index and foliage area index of rice using an indirect gravimetric method. Communications in Soil Science and Plant Analysis 45(13) 1726-1740. doi: 10.1080/00103624.2014.907917

Castilla LA, Pineda D, Ospina J, Echeverry J, Perafan R, Garces G, Sierra J and Dias A. 2010. Cambio climático y producción de arroz. Revista Arroz 58(489): 4–11.

Chen CC, McCarl BA and Schimmelpfennig DE. 2004. Yield variability as influenced by climate: A statistical investigation. Climatic Change 66(1-2): 239-261. doi: 10.1023/B:CLIM.0000043159.33816.e5

Datt I, Mehla BS, Goyat B and Kaliramana RS. 2012. Variability and correlation coefficient analysis of plant height, yield and yield components in rice *(Oryza sativa* L.). Annals of Biology 28(1): 45-52.

Díaz S, Pérez N and Morejón R. 2000. Evaluación Del Germoplasma De Arroz (*Oryza sativa* L). Cultivos Tropicales 21(2): 5-8.

De Costa WAJM, Weerakoon WMW, Herath HLMJ, Amaratunga KSP and Abeywardena RMI. 2006. Physiology of yield determination of rice under elevated carbon dioxide at high temperatures in a subhumid tropical climate. Field Crops Research 96(2-3): 336-347. doi: 10.1016/j.fcr.2005.08.002

Degiovanni V, Martínez CP, César P and Motta F. 2010. Producción eco-eficiente del arroz en América Latina. First Edition. Centro Internacional de Agricultura Tropical, Cali. 487 p.

Delerce S, Dorado H, Grillon A, Rebolledo MC, Prager SD, Patiño V, Gárces G and Jiménez D. 2016. Assessing weather-yield relationships in rice at local scale using data mining approaches. PLoS ONE 11(8) e0161620. doi: 10.1371/journal.pone.0161620

Dong H, Chen Q, Wang W, Peng S, Huang J, Cui K and Nie L. 2017. The growth and yield of a wet-seeded rice-ratoon rice system in central China. Field Crops Research 208: 55–59. doi: 10.1016/j.fcr.2017.04.003

Fageria NK. 2007. Yield physiology of rice. Journal of Plant Nutrition 30(6): 843-879. doi: 10.1080/15226510701374831

FAO. 2017. Country Indicators. In: FAOSTAT, http://www.fao.org/home/en/.; accessed: January 2018.

Fu JD and Lee BW. 2008. Changes in photosynthetic characteristics during grain filling of a functional stay-green rice SNU-SG1 and its F_1 hybrids. Journal of Crop Science and Biotechnology 11(1): 75-82.

Garcés G and Restrepo H. 2015. Growth and yield of rice cultivars sowed on different dates under tropical conditions. Ciencia e Investigación Agraria 42(2): 8-8. doi: 10.4067/S0718-16202015000200008

Geladi P and Kowalski BR. 1986. Partial least-squares regression: a tutorial. Analytica Chimica Acta 185: 1-17. doi: 10.1016/0003-2670(86)80028-9

Hastie T, Tibshirani R and Friedman J. 2009. The Elements of Statistical Learning. Second Edition. Springer Science+Business Media, New York. 745 p. doi: 10.1007/b94608

Hidayati N, Triadiati and Anas I. 2016. Photosynthesis and transpiration rates of rice cultivated under the system of rice intensification and the effects on growth and yield. HAYATI. Journal of Biosciences 23(2): 67-72. doi: 10.1016/j.hjb.2016.06.002

Huang M, Shan S, Cao F and Zou Y. 2016. The solar radiation-related determinants of rice yield variation across a wide range of regions. NJAS - Wageningen Journal of Life Sciences 78: 123-128. doi: 10.1016/j.njas.2016.05.004

Iizumi T, Luo J, Challinor A, Sakurai G, Yokozawa M, Sakuma H, Brown M and Yamagata T. 2014. Impacts of El Niño Southern Oscillation on the global yields of major crops. Nature Communications 5(3712). doi: 10.1038/ncomms4712

IPCC. 2014. Cambio climático 2014: Informe de síntesis - Resumen para responsables de políticas. In: The Intergovernmental Panel on Climate Change, https://www.ipcc.ch. 33 p.; accessed: January 2018.

Jarma A, Cardona C and Araméndiz H. 2012. Efecto del cambio climático sobre la fisiología de las plantas cultivadas: Una Revisión. Revista U.D.C.A Actualidad & Divulgación Científica 15(1): 63-76.

Ko J, Kim HJ, Jeong S, An JB, Choi G, Kang S and Tenhunen J. 2014. Potential impacts on climate change on paddy rice yield in mountainous highland terrains. Journal of Crop Science & Biotechnology 17(3): 117-126. doi: 10.1007/s12892-013-0110-x

Lancashire PD, Bleiholer H, Boom T, Langelüddeke P, Stauss R, Weber E and Witzenberger A. 1991. A uniform decimal code for growth stages of crops and weeds. Annals of Applied Biology 119(3): 561–601. doi: 10.1111/j.1744-7348.1991.tb04895.x

Mae T, Inaba A, Kaneta Y, Masaki S, Sasaki M, Aizawa M, Okawa S, Hasegawa S and Makino A. 2006. A large-grain rice cultivar, Akita 63, exhibits high yields with high physiological N-use efficiency. Field Crops Research 97(2-3): 227–237. doi: 10.1016/j.fcr.2005.10.003

Peng S, Khush GS, Virk P, Tang Q and Zou Y. 2008. Progress in ideotype breeding to increase rice yield potential. Field Crops Research 108(1): 32-38. doi: 10.1016/j.fcr.2008.04.001

Sameera S, Srinivas T, Rajesh A, Jayalakshmi V and Nirmala P. 2016. Variability and path co-efficient for yield and yield

components in rice. Bangladesh Journal of Agricultural Research 41(2): 259-271.

Sánchez AD, Garcés and Restrepo H. 2014. Biochemical and physiological characterization of three rice cultivars under different daytime temperature conditions. Chilean Journal of Agricultural Research 74(4): 373-379. doi: 10.4067/S0718-58392014000400001

Setter TL, Laureles EV and Mazaredo AM. 1997. Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. Field Crops Research 49(2-3): 95-106. doi: 10.1016/S0378-4290(96)01058-1

Song Q, Zhang G and Zhu XG. 2013. Optimal crop canopy architecture to maximise canopy photosynthetic CO_2 uptake under elevated CO_2 A theoretical study using a mechanistic model of canopy photosynthesis. Functional Plant Biology 40(2): 109-124. doi: 10.1071/FP12056

Thippani S, Kumar SS, Senguttuvel P and Madhav MS. 2017. Correlation Analysis for Yield and Yield Components in Rice (*Oryza sativa* L.). International Journal of Pure & Applied Bioscience 5(4):1412-1415. doi: 10.18782/2320-7051.5658

Ying J, Peng S, He Q, Yang H, Yang C, Visperas RM and Cassman K. 1998. Comparison of high-yield rice in tropical and subtropical environments I. Determinants of grain and dry matter yields. Field Crops Research 57(1): 71-84. doi: 10.1016/S0378-4290(98)00077-X

Yoshida S. 1981. Fundamentals of Rice Crop Science. Los Baños, Filipinas. The International Rice Research Institute, Los Baños. pp. 65-109.

Yoshida S and Parao FT. 1976. Climatic influence on yield and yield components of lowland rice in the tropics. Climate and rice 20: 471-494.

Zhang Y, Tang Q, Zou Y, Li D, Qin J, Yang S, Chen L, Xia B and Peng S. 2009. Yield potential and radiation use efficiency of "super" hybrid rice grown under subtropical conditions. Field Crops Research 114(1): 91-98. doi: 10.1016/j.fcr.2009.07.008

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Vulnerability to climate change of smallholder cocoa producers in the province of Manabí, Ecuador

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Vulnerabilidad al cambio climático de pequeños productores de cacao en la provincia de Manabí, Ecuador

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ABSTRACT

The consequences of climate change in the agricultural sector worldwide expose the need to understand the scope of their impact in order to develop mitigation and adaptation strategies for them. Therefore, this research evaluated the alterations in the environmental conditions and their relation with the vulnerability of smallholder cocoa (*Theobroma cacao* L.) producers to climate change in the province of Manabí. A non-probabilistic sampling of 1,060 small farmers was made in five cantons of Manabí. The vulnerability was determined through indicators such as the normalized difference vegetation index (NDVI), deforestation data from 1990 to 2016, models of the changes in climate and extreme weather events, satellite images, records from the National Institute of Meteorology and Hydrology (INAMHI by its initials in Spanish), and numerical outputs of mathematical models calibrated for Ecuador climatic and environmental data. Each indicator was calculated in conventional units and then categorized into vulnerability levels: low, medium, high and very high. For the indicators' superposition, algebraic tools of the Geographic Information Systems' (GIS) maps were used. The results showed a very high incidence of extreme events, deforestation higher than 6,000 ha year^{-1}, an increase of 0.8 °C in temperature between 1960 and 2006, an increase in rainfall on the coastal zone close to 90% and a decrease of it of more than 20% on the agricultural area. Furthermore, coverage showed the following distribution of the determined vulnerability levels: low (13.30%), medium (34.74%), high (45.53%), and very high (6.43%).

Keywords:
Coverage
Deforestation
Extreme weather events
Rainfall
Temperature
Theobroma cacao L

RESUMEN

Las consecuencias del cambio climático en el sector agrícola a nivel mundial dejan expuesta la necesidad de entender los alcances de su impacto para así desarrollar estrategias de mitigación y adaptación para las mismas. Por lo tanto, esta investigación evaluó las alteraciones en las condiciones ambientales y su relación con la vulnerabilidad que tienen los pequeños productores de cacao (*Theobroma cacao* L.) al cambio climático en la provincia de Manabí. Se hizo un muestreo no probabilístico de 1.060 pequeños agricultores en cinco cantones de Manabí. La vulnerabilidad se determinó mediante indicadores como el índice de vegetación de diferencia normalizada (NDVI por sus siglas en inglés), datos de deforestación de 1990 a 2016, modelos de los cambios en el clima y eventos climáticos extremos, imágenes de satélite, registros del Instituto Nacional de Meteorología e Hidrología (INAMHI) y salidas numéricas de modelos matemáticos calibrados para datos climáticos y ambientales del Ecuador. Cada indicador se calculó en unidades convencionales y luego se categorizó en niveles de vulnerabilidad: baja, media, alta y muy alta. Para la superposición de los indicadores se usó herramientas algebraicas de los mapas del Sistema de Información Geográfica (SIG). Los resultados muestran una incidencia muy alta de eventos extremos, deforestación superior a 6.000 ha año^{-1}, un incremento de la temperatura en 0,8 °C de 1960 a 2006, un aumento en las precipitaciones en la zona costera cercano al 90% y una disminución en las mismas superior al 20% en la zona agrícola. Además, la cobertura arrojo la siguiente distribución en los niveles de vulnerabilidad: baja (13,30%), media (34,74%), alta (45,53%), y muy alta (6,43%).

Palabras clave:
Cobertura
Deforestación
Eventos climáticos extremos
Precipitaciones
Temperatura
Theobroma cacao L

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According to the IPCC (2014), the temperature of the planet's surface has increased by approximately 0.2 °C per decade since the 1980s; however, this phenomenon has been accelerating since the end of 1990. Likewise, the projections of mathematical climate models show increases to 2 °C in 2050 and up to 3 °C at the end of the century.

The studies of the International Center for the Investigation of the El Niño Phenomenon CIIFEN (2014), and Thieelen *et al.* (2015), have determined that the changes will be gradual and will be accompanied by an increase in climatic variability and extreme events, which will generate more frequent episodes of droughts and floods, as well as an increase in the intensity of rainfall. In Ecuador, future scenarios show increases in temperature in the coastal region and reductions in the precipitation in the northern center of the Manabí province where precisely much of the agricultural activity takes place, and water sources play an important role because they are mainly used for irrigation, human consumption, and hydroelectric energy generation (Muñoz, 2010).

According to Vergara *et al.* (2014), climate change has strong effects on agricultural activities. Considering that cocoa crops are susceptible to changes in environmental conditions, the occurrence of this variation has adverse effects on it. These extreme phenomena could cause an alteration in the development stages and rates of pests and diseases related to cocoa, a decrease in the incubation periods and development of harmful organisms, and high ease of introduction of invasive species as well as changes in their geographical distribution (Schroth *et al.*, 2016). Cocoa crops' productivity and all the socioeconomic variable related to it can be severely affected if those prone evens happen; therefore, it is possible that 580,000 ha dedicated to the production of cocoa (*Theobroma cacao* L.) in Ecuador will be at risk. Approximately 60% of that agricultural area correspond to smallholder producers that have less than 3.5 ha each, and whose total cocoa production generate 820 million dollars of earnings to the country which equivalent to 0.6% of GDP (ANECACAO, 2015).

Recent research found tremendous effects on cocoa cultivation due to drought events, reporting losses in production yields between 10 and 46% in Indonesia (Schwendenmann *et al.*, 2010). Gateau-Rey *et al.* (2018) found in farms, chosen randomly in Brazil, a high mortality of cocoa trees (15%) and a severe decrease in cocoa yield (89%), as well as an increase in the rate of infection of the chronic fungal disease *Moniliophthora perniciosa* after the environmental conditions imposed by the Niño phenomenon between 2015 and 2016,. These findings, in the opinion of the authors, demonstrate that cocoa producers are at risk, and the increasing frequency of strong weather events will likely cause a decline in cocoa yields in the coming decades. Besides, cocoa and other crops can be the warning of the next important effects of the climate change on the natural and semi-natural vegetation.

Regarding the Ecuadorian outlook, the MAGAP (2015) reported that yields in cocoa production systematically had been increasing since 2002. Going from a national average of 0.17 to 0.60 t ha^{-1}. Only a shrinkage has been reported in 2012 that registered a 40% decrease in the total production (133 t) compared to 2011 (230 t), which undoubtedly is related to the occurrence of the La Niña Phenomenon in the South American coast between 2010 and 2011, generating strong droughts with a devastating effect on agricultural activities.

Although there are reports about annual cocoa yields, there are no studies that show the relationship between climate change and the alteration in the environmental conditions with the production of cocoa (*T. cacao* L.). Therefore, this research evaluated the alterations in the environmental conditions and their relation with the vulnerability of smallholder cocoa (*Theobroma cacao* L.) producers to climate change in the cantons of Chone, Tosagua, Bolívar, Junín, and Portoviejo in the province of Manabí. The vulnerability was determined based on the indicators such as the normalized difference vegetation index (NDVI), deforestation data from 1990 to 2016, models of the changes in climate and extreme events, satellite images, records from the National Institute of Meteorology and Hydrology (INAMHI by its initials in Spanish), and numerical outputs of mathematical models calibrated for Ecuador climatic and environmental data. Each indicator was calculated in conventional units and then categorized into vulnerability levels: low, medium, high and very high.

MATERIALS AND METHODS

The study was carried in a total area of 2,134.50 ha in the cocoa agricultural regions of Chone (0°37'59.5"S, 79°55'17.7"W), Bolívar (0°50'31"S, 80°09'43"W), Tosagua (0°47'02.2"S, 80°14'06.8"W), Junin (01°01'20.2792"S, 080°27'38.5844"W), and Portoviejo (1°01'20.3"S, 80°27'38.6"W) in the province of Manabí, Ecuador. A non-probabilistic sample of 1,060 smallholder farmers was used, these cocoa producers were selected because they have territory extensions of less than 5 ha and are certified as organic cocoa producers. Unstructured surveys and interviews were carried out to verify the information; the collected data was complemented with the direct observation on the premises and interviews of technicians of governmental institutions and advisers of rural producers.

For the chosen cocoa agricultural areas, their vulnerability to climate change was determined through several climatic and environmental indicators such as the Normalized Difference Vegetation Index (NDVI), the deforestation coverage, the occurrence of extreme weather events, the models of climate changes as well as the superposition of the digital information of the same. Besides, the vulnerability was classified into four levels: very high, high, medium and low.

Generation of indicators based on satellite data

The satellite information and its geometric corrections were made according to the methodology proposed by Montilla Pachecho and Pacheco Gil (2017) in order to process the image of the Landsat 8 sensor, LC80110612016332LGN00, from November 2016. The objective of this procedure is to orient the pixel positions regarding Ecuador's cartographic reference system through control points (GCP).

Radiometric corrections

The methods that tend to eliminate dispersion by subtraction were applied in order to approximate the response received by the sensor with the real object observed on the earth's surface. The method used in this phase was the minimum histogram. According to Hum *et al.* (2014), it is limited to subtract in each band the minimum value observed since it assumes that in a scene there can be some pixels in total shadow, which in the absence of atmosphere would and should not reflect any solar energy. With these procedures, more accurate data were obtained than previous versions of Landsat, incorporating substantial improvements in geometrical and radiometric aspects, according to the studies of Mishra *et al.* (2016), Sousa and Small (2017), and Roy *et al.* (2016).

Calculation of the Normalized Difference Vegetation Index (NDVI)

This index was determined according to the approaches of Baihua and Burgher (2015), to determine the characteristics of the vegetation in semi-arid zones in ecological conditions similar to those present in the studied places. The NDVI is obtained with the equation:

$$NDVI = \frac{(NIR - VISR)}{(NIR + VISR)}$$

Where:
NIR: Near-infrared band.
VISR: Red band

With the application of the above equation, an image with normalized magnitudes between -1 and 1 was generated, where the negative values and close to 0 indicate zones devoid of vegetation, and those leading to 1 indicate areas with very dense cover vegetation. This coverage was reclassified into four vulnerability levels through a spatial distribution analysis (Table 1).

Coverage of deforestation

The historical deforestation information of the MAE (2017) was used for the periods 1990-2000, 2000-2008, 2008-2014, and 2014-2016. With the Geographic Information Systems (GIS) the four coverages (bare soil, dry forest, transition forest, and humid forest) were united in a single map, which was reclassified in a Boolean image with low and very high vulnerability levels for the zones without deforestation and with deforestation, respectively.

Extreme weather events and models of climate changes

The historical data of the INAMHI were used to define the affectation due to extreme weather events, considering as extreme the cases above the last quartile and below the first one of the series of data for the period 1970-2016. On the other hand, the proposal of

Schroth *et al.* (2016) was considered for the models of climate changes, processing the numerical outputs generated by the MAE (2017) and INAMHI (2017) with the ETA and TL959 models, these models presented the best correlations with the historical records for precipitation and temperature, respectively. Shapefiles were constructed with the information obtained from these models, with the coverage of changes in precipitation and temperature for the province of Manabí; categorizing the vulnerability into four levels according to the climate elements in very high, high, medium and low.

RESULTS AND DISCUSSION

The Normalized Difference Vegetation Index (NDVI) reported values ranging from -0.278 to 0.549, with an average of 0.212. Coverage of transition and dry forest comprised about 70.35% of the territory studied and had a medium and high vulnerability, respectively (Table 1). Although the humid forest presented a low vulnerability when the experiment was carried out, it is an area that must be monitored since it could be potentially used for agriculture expansions to sow cocoa and other crops. It is notable that this area represented more than 25% of the studied zone.

Table 1. Categorization of vulnerability according to the normalized difference vegetation index.

NDVI	Surface		Vulnerability level	Type of coverage
	(km^2)	(%)		
0.00-0.10	99.24	3.25	Very high	Bare soil
0.10-0.18	904.09	29.58	High	Dry forest
0.18-0.27	1,246.23	40.77	Medium	Transition forest
>0.27	779.175	25.49	Low	Humid forest
<0.00	279.504	0.91	Null	Water bodies

The search for new sites suitable for producing cocoa could trigger the clearing of forests and natural protected areas, which are important for flora and fauna habitats (Ruf *et al.*, 2015). In this sense, Turbay *et al.* (2014) recommend adequate shade management in crops, renewal with disease-resistant varieties, crop association, plant cover, stepped sowing, and reforestation as strategies to reduce vulnerability to climate change. As Figure 1 shows, the vulnerability levels very high and high are mostly located in areas close to the coast, where dry forest coverage predominated; while low vulnerability was distributed in the central western mountainous area with predominance of the humid forest coverage.

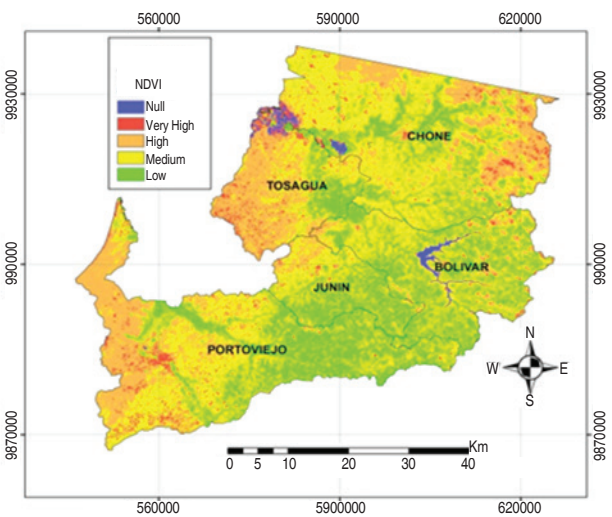

Figure 1. Spatial distribution of vulnerability in response to the normalized difference vegetation index (NDVI).

Deforestation

Table 2 and Figure 2 show the concentration of the deforested areas in the northern part of the studied zone which belong to Chone and Bolívar cities, encompassing a total deforested area of 392.06 km^2 (12.8% of the studied area) between 1990 and 2016, with a deforestation rate higher than 1,500 ha year^{-1}. High deforestation is attributed to changes in land use, generally for agricultural activities such as mainly extensive cattle-breeding and short-cycle crops. The practice of these activities with technologies that are not friendly to the environment increases vulnerability to climate change because it favors the emission of greenhouse gases and the loss of forest and soil

Table 2. Deforestation during the period 1990-2016.

Deforestation Period (1990-2016)	Surface		Vulnerability Level
	(km^2)	%	
Forested area	2,661.18	87.16	Low
Deforested area	392.06	12.84	Very high

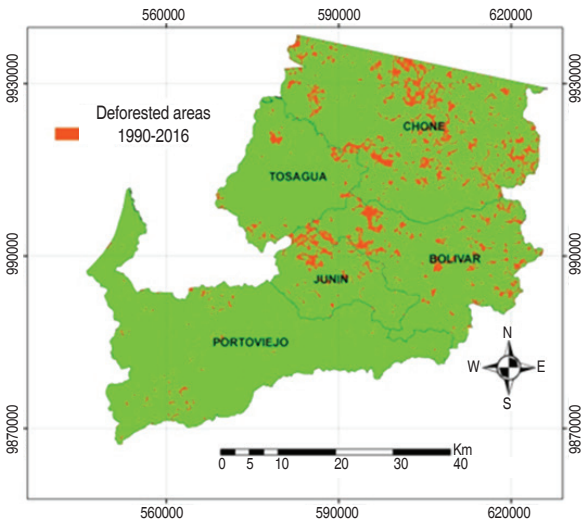

Figure 2. Spatial distribution of deforestation due to agricultural activities between 1990 and 2016.

resources (MAE, 2017). This information is consistent with studies conducted in West Africa, reported by Ruf *et al.* (2015), where cocoa cultivation is indicated as an important deforestation driver. On-site detection for replanting old plantations, farmers usually migrated to the forest borders to establish new cocoa farms in order to find more humid areas to sow it.

Deforestation has a direct relation to climate change. Forest degradation leads to the loss of carbon dioxide repositories. The carbon storage compartments in trees are aerial biomass, mainly in the trunks of woody plants and leaves; there is also underground biomass that stores it in the root tree network. The carbon accumulated by trees, and hence by the forest, can move and be stored in the soil due to the necromass and plant litter.

The main tool to prepare agricultural land is the burning of green areas and forest; this practice leads to increase deforestation. Besides, scorched vegetation releases CO_2, CH_4, N_2O, ozone precursors, and aerosols (including black carbon) to the atmosphere. The vegetation that develops after a fire is going to absorb and consume

atmospheric CO_2 and nitrogen. Anthropogenic land management or land transformation through fire leads to an increase in the levels of disturbance or permanent clearance of forest. This action results in net emissions to the atmosphere over time. Satellite detection of fire occurrence and persistence has been used to estimate fire emissions. However, it is hard to separate the source of fire as natural or anthropogenic. These conditions are intensified in the studied area due to an average of 400 kg of nitrogen fertilizer is used per extension and livestock is produced with a loading capacity of only 1 head ha^{-1}, wherewith the great influence of deforestation on the land of smallholder cocoa producers in the Province of Manabí was determined.

Fieldwork carried out in the studied area, and the use of dated satellite images interception updated how is the processes of continuous deforestation with current trends – an estimation of 4,000 ha $year^{-1}$ in the rural territory, which represents approximately 20.00 km^2. However, these data do not represent facts. The Ecuadorian government ratified the commitments made in the Paris agreement and presented a zero deforestation goal for the year 2025. Public policies and citizen training must be implemented to achieve the objectives, which imply significant changes in the current technologies production and incorporation of sustainable mechanisms economically and environmentally speaking.

Extreme weather events

The thermal and rainfall regimes in the central region of the Manabí province are characterized by a high seasonality, related to the seasonal warming of the equatorial Pacific and the displacement of the intertropical convergence zone (Thielen *et al.*, 2015). During this seasonality, anomalies with a high incidence of extreme weather events such as La Niña and El Niño phenomenon occur. These phenomena produce two extreme conditions such as intense periods of drought or rainfall, the occurrence of one of them of depending on the geographical zone that they occur. Therefore, cocoa crops are susceptible to these both weather extreme event (Ojo and Sadiq, 2010).

The analysis of climate data shows that the frequency of positive and negative thermal anomalies (El Niño and La Niña), which generate extraordinary rainfall and drought is very high in the equatorial Pacific. Therefore, the entire coastal area of Ecuador has a very high vulnerability to extreme events of this type (Figure 3).

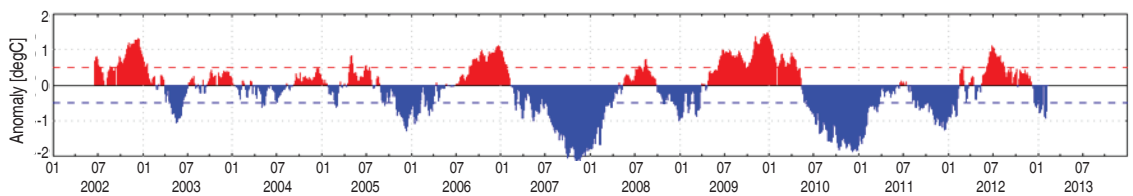

Figure 3. Thermal anomalies in the sea surface temperature of the Equatorial Pacific Ocean between 2002 and 2012.

During 2015, 2016, and 2017 the extraordinary rains, related to the anomalous warming of the sea surface in the equatorial Pacific coast, altered the historical averages in the precipitations, the solar brightness, and the environmental temperature causing severe impacts in the flowering, the ears' development, and the growth of the cacao trees (MAGAP, 2015). The rainfall in the Ecuadorian coastal area has exceeded, in some cases, more than 500% of the historical averages (INAMHI, 2017).

Regarding the incidence of these climatic alterations in cocoa cultivation, Schrot *et al.* (2016) report that the cocoa fruit does not develop completely during the droughts, and very intense rains diminish the flowering and the fruit set; hence, both events reduce cocoa productivity. On the other hand, in response to the increase in temperature, the cacao trees restrict the development of pods to get the most water for growth. MAGAP reported the increasing incidence of the monk because of the El Niño phenomenon in 2017. It is known this pest occurs in rainy seasons, where the temperature and humidity conditions are favorable for the growth of the fungus *Moniliophthora roreri* which causes watery cocoa rot (INIAP, 2015).

Considering environmental conditions for the criteria the normal development of cocoa cultivation occur at temperatures between 18 and 34 °C and precipitations per cycle from 1200 to 3000 mm are required (INIAP, 2015). The average monthly precipitation range required for cocoa cultivation is 125 mm. When rainfall does not cover water needs, farmers must use irrigation (in areas with water availability) to compensate the deficit and avoid production losses. The entire province was categorized in a very high vulnerability to cocoa cultivation considering the high frequency in the occurrence of extreme events.

Gateau-Rey *et al.* (2018) reported severe decreases in the soil's water content, because of the extreme weather events caused by the El Niño phenomenon in Brazil from 2015 to 2016. The water deficit of the soil has a great influence on the yield decrease of the cocoa crop.

Model of the climate changes

The decrease in precipitation is located precisely in the mountainous zones recognized as water-producing areas (Figure 4); therefore, this decrease has a direct effect on the availability of water for future irrigation systems in cocoa areas since the monthly rainfall average in them barely exceeds the amount required by the crop (125 mm year^{-1}). On the other hand, the increase in temperature would affect the availability of water due to the increase in evapotranspiration although it would not surpass the optimal temperature limits of cocoa crops (Muñoz, 2010).

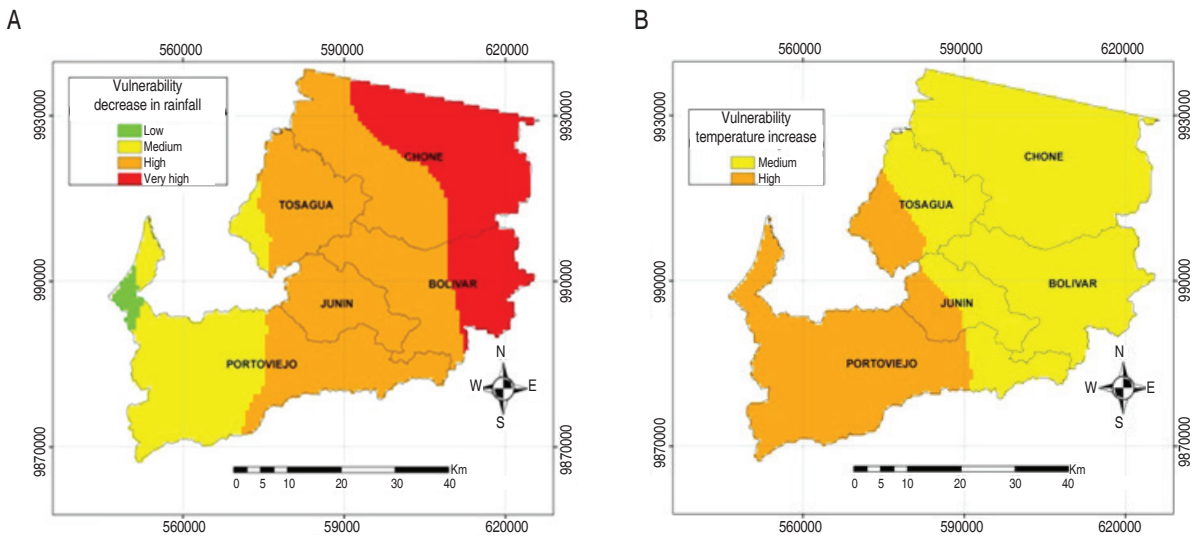

Figure 4. Vulnerability according to A. Decrease in rainfall; B. Temperature increase.

The effects related to extreme events in the climate could generate great consequences for the global production of cocoa, not only because of the physiology of the plant but also because of the increase of diseases and pests (Ruf *et al.*, 2015).

The categorization of vulnerability due to climatic events showed that 34.69% of the studied area has a high vulnerability to temperature variations, with increases of up to 0.8 °C (Table 3). On the other hand, 54.51% of the territory is highly vulnerable to the decrease in rainfall levels, with negative values between 40 and 50% of the total annual rainfall; that variation means that the crop could be affected since the lack of water affects the floral development.

The results of the model of climate changes suggest the loss of environmental capacity for the cocoa cultivation in the coast, the increase of the temperature would displace the crop areas to higher altitudes. This situation is contrary to the results found by the CIAT (2014) for Ecuador's Andean region, where cocoa crops move to lower areas.

As a strategy to face the problem, it is necessary to update the information with research on irrigation

systems suitable for cultivation. Parallel to it, there should be formulated government policies to help farmers and the cocoa industry to prepare for facing and adapting to climate change.

Table 3. Vulnerability categorization according to thermal and rainfall anomalies.

Thermal elevation (ºC)	Surface		Decrease in rainfall (%)	Surface		Vulnerability level
	(km²)	(%)		(km²)	(%)	
>0.8	0	0	>50	724.24	23.81	Very high
0.4-0.8	1,058.6	34.69	40-50	1,658.26	54.51	High
0.2-0.4	1,994.1	65.31	30-40	627.78	20.64	Medium
<0.2	0	0	<30	31.63	1.04	Low

Vulnerability summary

The overlapping of the indicators reflects the total vulnerability in four levels (Table 4), where most of the areas evaluated are categorized into a high and very high vulnerability, they together account for more than 50% of the territory. This result implies urgent needs to implement mitigation and adaptation measures to climate change.

Table 4. Categorization of the total vulnerability.

Surface		Vulnerability level
(km²)	(%)	
190.53	6.43	Very high
1,349.29	45.53	High
1,029.64	34.74	Medium
394.05	13.30	Low

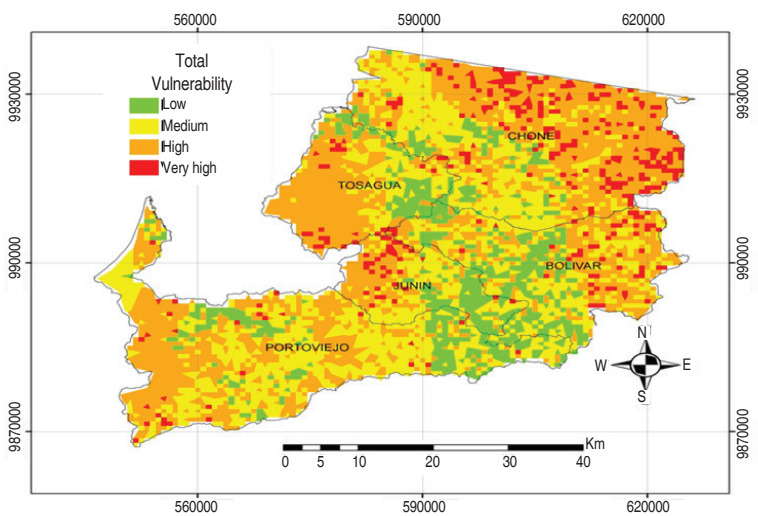

Figure 5. Spatial distribution of the total vulnerability.

This study also specified the particularly vulnerable areas (Figure 5) to lead decision making by cocoa producers in these areas. They need to implement strategies for the adaptation and mitigation to bear the climate change, which will allow them to enrich or maintain the productivity of the cocoa crops. Apply those measures is not possible if they do not possess the knowledge, the tools, and the support of governmental and institutional entities. Nowadays, several alternatives can be implemented such as agro-tourism, the integration of family labor, community and union associations, day labor, and marketing strategies; the latter alternative encompasses fair markets and product certifications that help to improve sales prices and withstand the moments of crisis. These strategies can be adapted to the cultivation of cocoa in the studied area.

CONCLUSIONS

The indicators analyzed yielded that the normalized vegetation difference index groups 34% of the studied territory into high and very high vulnerability to climate change. Besides, 12% of the territory presents deforestation, going from forest cover to agricultural mosaic, due to unsustainable agricultural practices regarding the climate effects. The province of Manabí is exposed to frequent extreme weather events such as droughts and floods which is evidenced by the anomalies of the sea surface temperature; actually, 77% of its territory presented high and very high vulnerability to the models of the climate changes. The combined action of all the studied indicators generates high and very high vulnerability in 52% of the territory analyzed. These results suggest the need to consider scenarios for the implementation of adaptation and mitigation measures that increase the resilience of populations and ecosystems to the effects of climate change.

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REFERENCES

ANECACAO. 2015. Exportaciones de Cacao del Ecuador. In: Asociación Nacional de Exportadores de Cacao, http://www.anecacao.com/index.php/es/inicio.html. 6 p.; accessed: January 2018.

Baihua F and Burgher I. 2015. Riparian vegetation NDVI dynamics and its relationship with climate, surface water and groundwater. Journal of Arid Environments 113: 59-68. doi: 10.1016/j.jaridenv.2014.09.010.

CIIFEN. 2014. Estrategia Provincial de Cambio Climático: Fase I: Diagnóstico. Implementación de un sistema de información de vulnerabilidad sectorial de la provincia del Guayas frente al cambio y la variabilidad climática. In: Centro Internacional para la Investigación del Fenómeno de El Niño, http://www.ciifen.org/. 95 p.; accessed: February 2018.

Ojo AD and Sadiq I. 2010. Effect of climate change on cocoa yield: a case of cocoa research institute (CRIN) farm, Oluyole local government Ibadan Oyo State. Journal of Sustainable Development in Africa 12(1): 350-358.

Gateau-Rey L, Tanner EVJ, Rapidel B, Marelli JP and Royaert S. 2018. Climate change could threaten cocoa production: Effects of 2015-16 El Niño-related drought on cocoa agroforests in Bahia, Brazil. Plos One 13(7): e0200454. doi: 10.1371/journal.pone.0200454

Hum YC, Lai KW and Mohamad Salim MI. 2014. Multiobjectives bihistogram equalization for image contrast enhancement. Complexity 20(2): 22-36. doi: 10.1002/cplx.21499

INAMHI. 2017. Anuario Meteorológico N° 53-2013. First Edition. Dirección ejecutiva del INAMHI, Quito. 144 p.

INIAP. 2015. Informe anual 2015. In: Instituto Nacional de Investigaciones Agropecuarias, http://repositorio.iniap.gob.ec/handle/41000/4094 104 p.; accessed: February 2018.

CIAT. 2014. Evaluación de la vulnerabilidad al cambio climático de la agricultura en la región Andina de Ecuador. In: Centro Internacional de Agricultura Tropical, http://dapa.ciat.cgiar.org/wp-content/uploads/AboutUs/PolicyBriefs/policy-brief-15-evaluacion-vulnerabilidad-cambio-climatico-ecuador.pdf. 6 p.; accessed: January 2018.

IPCC 2014. Quinto Informe de Evaluación del Grupo Intergubernamental de Expertos sobre el Cambio Climático. In: The Intergovernmental Panel on Climate Change, https://www.ipcc.ch/. 27 p.; accessed: November 2017.

MAE. 2017. Mapa histórico de la deforestación del Ecuador continental. In: Ministerio del Ambiente del Ecuador, https://docplayer.es/13530720-Mapa-historico-de-deforestacion-del-ecuador-continental.html 46 p.; accessed: December 2017

MAGAP 2015. Boletín situacional de Cacao 2014. In: Ministerio de Agricultura y Ganadería, https://www.agricultura.gob.ec/. 7 p.; accessed: September 2017.

Mishra N, Helder D, Barsi J and MarKham B. 2016. Continuous calibration improvement in solar reflective bands: Landsat 5 through Landsat 8. Remote Sensing of Environment 185: 7-15. doi: 10.1016/j.rse.2016.07.032

Montilla Pacheco A and Pacheco Gil HA. 2017. Comportamiento temporal y espacial del bosque ribereño en el curso bajo del río Portoviejo y la quebrada Chilán, provincia de Manabí, Ecuador. Revista Internacional de Contaminación Ambiental 33(1): 21-35. doi: 10.20937/rica.2017.33.01.02.

Muñoz AG. 2010. Validación y análisis de consenso de modelos de escenarios de cambio climático para Ecuador. Proyecto inamhi-mae-scn-praa-pacc. Centro de Modelado Científico (CMC) de La Universidad del Zulia, Maracaibo. 129 p.

Roy DP, Kovalsky V, Zhang HK, Vermote EF, Yan L, Kumar SS and Egorov A. 2016. Characterization of Landsat-7 to Landsat-8 reflective wavelength and normalized difference vegetation index

continuity. Remote Sensing of Environment 185: 57-70. doi: 10.1016/j.rse.2015.12.024

Ruf F, Schroth G and Doffangui K. 2015. Climate change, cocoa migrations and deforestation in West Africa: What does the past tell us about the future?. Sustainability Science 10(1): 101–111. doi: 10.1007/s11625-014-0282-4

Sousa D and Small C. 2017. Global cross-calibration of Landsat spectral mixture models. Remote Sensing of Environment 192: 139-149. doi: 10.1016/j.rse.2017.01.033

Schroth G, Läderach P, Martinez-Valle AI, Bunn C and Jassogne L. 2016. Vulnerability to climate change of cocoa in West Africa: Patterns, opportunities and limits to adaptation. Science of The Total Environment 556: 231-241. doi: 10.1016/j.scitotenv.2016.03.024

Schwendenmann L, Veldkamp E, Moser G, Hölscher D, Köhler M, Clough Y, Anas I, Djajakirana G, Erasmi S, Hertel D, Leither D, Leuschner C, Michalzik B, Propastin P, Tjoa A, Tscharntke T and van Straaten O. 2010. Effects of an experimental drought on the functioning of a cacao agroforestry system, Sulawesi, Indonesia. Global Change Biology 16(5): 1515–1530. doi: 10.1111/j.1365-2486.2009.02034.x

Thielen D, Cevallos J, Erazo T, Zurita I, Figueroa J, Quintero J, Matute N, Velásquez G and Puche M. 2015. Dinámica de eventos climáticos extremos en la cuenca del río Portoviejo, Manabí. Revista La Técnica 14: 80-91.

Turbay S, Nates B, Jaramillo F, Vélez JJ and Ocampo OL. 2014. Adaptación a la variabilidad climática entre los caficultores de las cuencas de los ríos Porce y Chinchiná, Colombia. Investigaciones Geográficas, Boletín del Instituto de Geografía 2014(85): 95-112. doi: 10.14350/rig.42298

Vergara W, Ríos AR, Trapido P and Malarín H. 2014. Agricultura y Clima Futuro en América Latina y el Caribe: Impactos Sistémicos y Posibles Respuestas. In: Banco Interamericano de Desarrollo, https://publications.iadb.org/en/publication/16673/agriculture-and-future-climate-latin-america-and-caribbean-systemic-impacts-and 15 p.; accessed: February 2018.

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Perception of giant African snail (*Achatina fulica*) in urban community from Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Percepción del caracol gigante africano (*Achatina fulica*) en una comunidad urbana de Colombia

doi: 10.15446/rfnam.v72n1.73085

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ABSTRACT

In Colombia, the control of giant African snail populations (*Achatina fulica*) has been enforced for the past eight years according to the Environmental, Housing, and Territorial Development Ministry (MAVDT from its initials in Spanish). During this period, the environmental authorities have carried out a series of campaigns for snail eradication and to raise awareness in the general community to involve it in the control of this invasive species. In order to inquire about the perception of the citizens of Cali, Colombia, have of the giant African snail and their role as primary stakeholders in the local control programs, a structured survey was done, and the dependency on the sociodemographic characteristics was established through Fisher's exact test. 316 people took part in the survey; over 80% of the individuals recognized the giant African snail and their form of control, but over 90% of them did not participate in the control campaigns. The perception of the giant African snail varied noticeably with the respondents' age and was independent of socio-economic and educational level. In conclusion, it was detected a solid dissociation between people and the actions carried out by the environmental authority. It is recommended to take into account people's concept of invasive species control in other cities as a fundamental instrument in the construction of a more dynamic and inclusive control model.

Keywords:
Control
Human dimension
Invasive species
Stakeholder

RESUMEN

En Colombia, el control de las poblaciones del caracol gigante africano (*Achatina fulica*) lleva ocho años de vigencia de acuerdo con el Ministerio de Ambiente, Vivienda y Desarrollo Territorial (MAVDT). Durante este tiempo, las autoridades ambientales han realizado una serie de jornadas de erradicación del caracol y concientización de la comunidad para involucrarla en el control de esta especie invasora. Con el fin de indagar en la percepción de los ciudadanos de Cali, Colombia, sobre el caracol gigante africano y su rol como actor principal en los programas de control local, se construyó una encuesta estructurada y se estableció la dependencia de las características sociodemográficas mediante la prueba exacta de Fisher. Participaron 316 personas en total; más del 80% de los individuos reconocen al caracol gigante africano y la forma de control, pero más del 90% no participa en las jornadas de erradicación. La percepción del caracol gigante africano cambió notablemente con la edad de los encuestados y es independiente del nivel socioeconómico y educativo. En conclusión, se detectó una fuerte disociación entre las personas y las acciones que realiza la autoridad ambiental. Se recomienda tener en cuenta el concepto de las personas en el control de otras especies invasoras en otras ciudades como un instrumento fundamental en la construcción de un modelo de control más dinámico y participativo.

Palabras clave:
Control
Dimensión humana
Especie invasora
Actor social

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A stakeholder is any individual, group or organization who is affected (positively or negatively) by invasive species, or who has the capacity to promote or limit the invasive species expansion, and its role in the management and control programs would be a crucial factor to reduce the effect of invasive species (Shackleton *et al*., 2018). In Colombia, the giant African snail (*Achatina fulica,* Bowdich 1822) was included in the list of exotics species (MAVDT, 2008), and in an international scenario is considered one of the most dangerous exotic invasive species in the world (Lowe *et al.,* 2000; de la Ossa *et al.,* 2017). *A. fulica* is a native snail from Eastern Africa; from where, it has spread over large part of the world's tropical region during the past two centuries, bringing negative consequences to ecosystems, economies, and public health (Raut and Barker, 2002; Thiengo *et al.,* 2007; de la Ossa *et al.,* 2017; Córdoba *et al.,* 2017; Patiño and Giraldo, 2017). In countries such as the United States, native species have been displaced by competition with *Achatina fulica* (Roda *et al.,* 2016). The species is widely known due to the damage it produces to crops in Ecuador, and the worldwide dispersion of the nematode, *Angiostrongylus cantonensis*, from China is attributed to the giant African snail (Castaño and García, 2014; Peng *et al.,* 2017).

Due to the high economic and environmental costs attributed to the giant African snail, different control strategies have been adopted (da Silva and Marques, 2017). These strategies have been classified into three main groups: physical, chemical, and biological. Unfortunately, all the control and eradication strategies implemented to stop the giant African snail expansion have not been able to contain it (Correoso, 2006; Garcés *et al.,* 2016; Patiño, 2018; Smith *et al*., 2013; Thiengo *et al*., 2007). For this reason, the concept of eradication in the action plan against giant African snail was replaced by the concept of management, and new strategies using a combination of alternative methods of control have emerged, local scientific studies about the snail natural history and direct interaction with the stakeholders, were designed in order to reduce the negative impact of this species (Balfour and Alli, 2014).

According to the invasive species definition adopted by the International Union for Conservation of Nature (IUCN), people are a key factor in distribution, establishment, and success of foreign species in ecosystems (Lowe *et al.,* 2000; Pereyra, 2016). Therefore, the management of any invasive species requires the correct articulation of the different sectors or "actors" of the affected community. In general, the community's perception about the giant African snail would be determined by the education level of the inhabitants, the intensity of the divulgation campaigns, the direction of the campaigns towards biological knowledge or control methods, and the impact that the mollusks have in their daily lives, among others (Rout *et al.,* 2014; Andreazzi *et al.,* 2017). Moreover, the active participation of stakeholders in the local management plans could reduce the amount of money invested to control this invasive species (Crowley *et al.,* 2017a, 2017b).

In Colombia, a regulatory ordinance for this snail control just was signed in 2011, by that year its presence had already been reported in ten departments of the country. This ordinance set the responsibilities and obligations that the regional and municipal environmental authorities had to implement (MAVDT, 2011; Giraldo *et al*., 2014). During the last ten years, *A. fulica* has been considered an invasive species in Colombia, the Regional Autonomous Corporations (CARs by its initials in Spanish) have developed control activities, financed scientific research, and carried out public citizen warning activities on the giant African snail in their respective regions (Garcés *et al*., 2016; Córdoba *et al.,* 2017; de la Ossa *et al.,* 2017).

Valle del Cauca is the department of Colombia where the greatest amount of research on the giant African snail encompassed the ecology, genetics, parasitology, and alternative control methods of the species have been conducted, followed by the departments of Sucre, Antioquia, and Santander. However, it has been widely suggested that the giant African snail problem has not yet been contained in these region (Giraldo *et al.,* 2014; Garcés *et al.,* 2016; de la Ossa *et al*., 2017; Patiño and Giraldo, 2017; Varela *et al*., 2017). For example, in the biggest urban center of Valle del Cauca, the city of Cali, the environmental authority started a giant African snail control campaign based in manual recollection since 2012. Despite this huge effort, the population of

A. fulica continue latent. Therefore, to evaluate the role of the people who live in Cali as a key stakeholder in the local control program, it was evaluated their perception about the giant African snail under the assumption that the public warning campaigns have effectively reached all sectors of the city and the results were contrasted with other similar studies on this species (Moreira *et al.,* 2012; Luizaga *et al.,* 2015; Sá *et al.,* 2016; Andreazzi *et al*., 2017).

MATERIALS AND METHODS

Studied area

The city of Cali is located in the southwest of Colombia. It is the administrative and financial center of the department of Valle del Cauca and one of the largest cities in Colombia with a population of around 2.5 million and a density of 51 homes per hectare. The urban area is divided administratively into 22 communes that encompass 248 neighborhoods (Alcaldía de Santiago de Cali, 2018) (Figure 1).

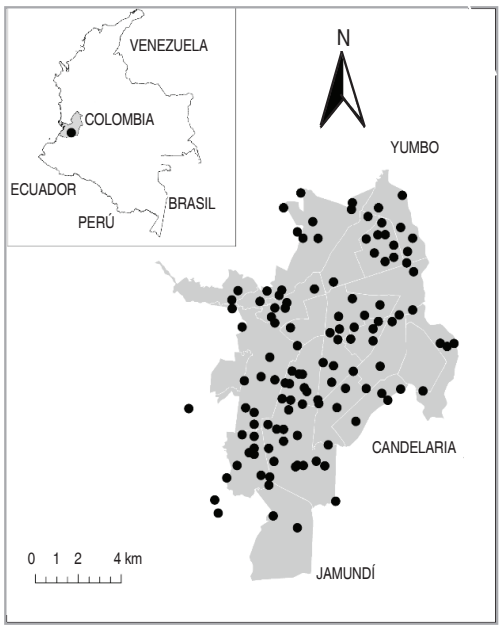

Figure 1. Studied area of Cali's urban zone and its administrative division by communes. Black points represent the respondents' location.

Designed questionnaire

A questionnaire was designed to collect information through a structured survey following the recommendations of García *et al.* (2008), Sá *et al.* (2016) and Andreazzi *et al.* (2017). The questionnaire had three sections; the first section contained questions related to socio-demographic characteristics such as home location, age, socio-economic stratum, and education level. The second section contained general questions on the invasive species, such as the concept of the species, negative consequences of its presence, the subject broadcasting, and environmental authorities in charge of dealing with the invasive species. The last section encompassed general information on the giant African snail, such as how to identify it, negative consequences of its presence, knowledge associated with parasites, control measures, participation in control efforts, and how the environmental authority efforts were perceived.

The questionnaire consisted of 23 open-ended and multiple-choice questions. The multiple-choice questions used language categories such as "Yes or No" or a scale of 1 to 10, where 1 was the minimum score and 10 the maximum score. The questionnaire was designed using a virtual application for its later broadcasting using digital and printed media. The statistical universe considered for census accuracy estimation was the population of Cali, and only one questionnaire per person was recorded. For the digital broadcasting, the electronic address of the questionnaire was divulged on social media and by email between July 2017 and February 2018, reaching 1,200 people. The following criteria were used to stop receiving

questionnaire responses: (i) Obtaining the desired statistical sample, 385 answered surveys considering physical and digital broadcasting, corresponding to a 95% confidence level and 5% error, (ii) total coverage of the communities in the Cali's urban area, (iii) the distribution of surveys by neighborhoods in Cali, and (iv) the number of days without replies or with only one reply to digital questionnaires.

Statistical analysis

Responses to questionnaires were organized in a database, similar to a presence-absence matrix, discriminating each question and answers, with one corresponding to an answer to the question and zero for the other options. The geographic coordinates of respondents' homes were obtained manually with Google Earth® program; age was grouped in categories of 14 to 20, 21 to 30, 31 to 40, 41 to 50, and 50 and above. For giant African snail locations, the categories of habitat (green area, water sources, median strips, among others) and the percentage of each type were calculated considering the number of people reporting this species. The questions with responses from one to ten were grouped into three categories as follows: "low" when the answer ranged from one to three, "medium" when the answer ranged from four to seven, and "high" when the answer ranged from eight to ten. The general trend of perception variables was evaluated descriptively, and its dependency on socio-demographic characteristics was evaluated with a contingency analysis using Fisher's exact test in the STATA program version 14.

RESULTS AND DISCUSSION

A total of 316 surveys were processed, covering 137 neighborhoods of Cali's 21 communes. Fifty-seven percent of participants were from medium socio-economic level, 33% were from low, and less than 20% were from high. Most participants were female (57.6%) ranging in age from 21 to 30 years old (34.5%). There were responses from people ranging an education level from primary school to postgraduate studies, with an equal number of participants from public and private institutions. One of the main problems with invasive species management is that it usually focuses on administrative-economic costs and available scientific knowledge on the species and neglects the social context (García *et al.,* 2008; Crowley *et al.,* 2017b). The heterogeneity of the community sharing space with these species influences the execution of control plans, and therefore awareness must be accompanied by an exploration of knowledge appropriation and understanding of the general problem (Shackleton *et al.,* 2018). Table 1 represents the complexity of Cali's society and not only of a section of it, and this is relevant for detecting potential conflicts among social actors (Rout *et al.,* 2014; Andreazzi *et al*., 2017; Crowley *et al.,* 2017a; Waliczek *et al.,* 2017).

90% of interviewed people stated that they knew what an invasive species was, and 94% thought they were dangerous, but 81% had not witnessed a divulgation campaign on the issue. 61% of people did not know what to do if they found these species and 59% did not know to which environmental authority they should report the problem (Figure 2).

Concerning the giant African snail, 79% of participants considered they had a low to medium level of knowledge on the giant African snail. 83% of interviewees considered that the amount of information received was low to medium, whereas perception of the veracity of the information was homogeneously distributed over low, medium, and high levels. When asked about the work of the environmental authority in managing and controlling the species, 90% of people perceived it as low to medium, with a consensus on low performance of the authority (52%). People associated the species with environmental damage and harm to public health; over 55% of people considered that the species produces great damage, although the trend was towards greater harm to public health than to environmental damage. Despite, this preconception there was little knowledge on the parasites associated with the mollusk, and 65% of people admitted not knowing on the associated parasites for which the giant African snail is considered dangerous to public health.

Most scientific researchers on the relationship between people – giant African snail suggest a negative perception and a low knowledge about the snail by the studied community (Moreira *et al.,* 2012; Luizaga *et al.,* 2015; Sá *et al.,* 2016; Andreazzi *et al.,* 2017). The same trend was established for the city of Cali. However, knowledge in people about control types methods and basic biological aspects of the species was adequate. Therefore, the lack of access to this knowledge should not be the cause of little active participation in control activities (Rout *et al*., 2014). Unfortunately, this little participation of the people in control activities tended to reduce the effectivity of management policies and limits the viability of alternative and inclusive methods of control (Garcés *et al.,* 2016; Shackleton *et al.,* 2018).

Table 1. Percentage of socio-demographic characteristics of people interviewed on perceptions of the giant African snail (*A. fulica*) in the city of Cali.

Variable	Category	Participants (n=316)	Frequency (%)
Gender	Female	182	57.6
	Male	134	42.4
Age	14-20	44	13.9
	21-30	109	34.5
	31-40	48	15.2
	41-50	52	16.5
	>50	62	19.6
	No inform	1	0.3
Socioeconomic level	Low	84	26.6
	Medium	180	57.0
	High	52	16.5
Educative level	Elementary	7	2.2
	High school	82	26.0
	Technician	74	23.4
	Undergraduate	92	29.1
	Postgraduate	61	19.3
Educational institution	Public	152	48.1
	Private	153	48.4
	No inform	11	3.5

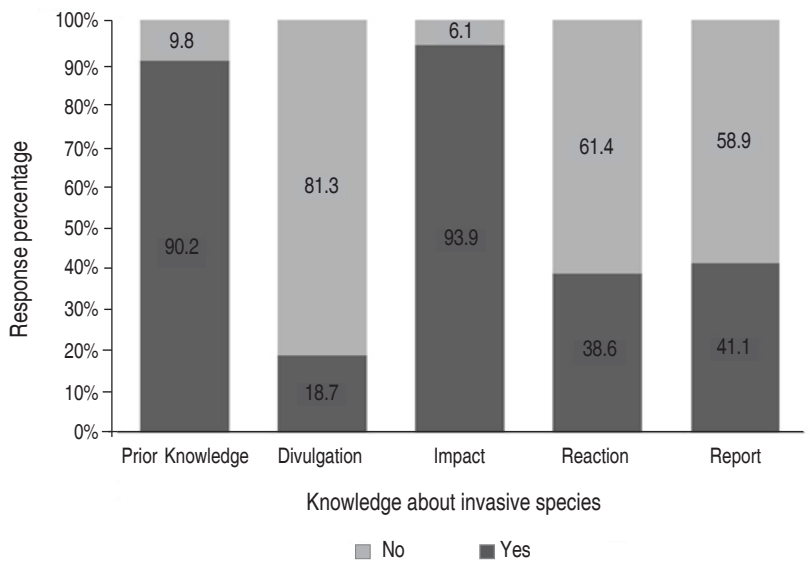

Figure 2. Knowledge perception of invasive species by Cali's interviewed people (n=316).

69% of the people surveyed indicated that they do not know how to manipulate and dispose the individuals of giant African snail when they detect them in their neighborhood. Moreover, 94% of interviewees had

never participated in control efforts, either by their own initiative, by the community association, or by the environmental authority, and 88% stated that they have not observed another invasive species besides the giant African snail (Figure 3). Most people have observed the giant African snail associated with green areas (61%) or gardens (14%), although their presence on crops and even as pets in rural areas was also mentioned. Finally, 73% of the interviewees stated that they do not know if nearby people have had situations with the giant African snail, and 88% of interviewees did not know if there is another species of invasive snail in Colombia.

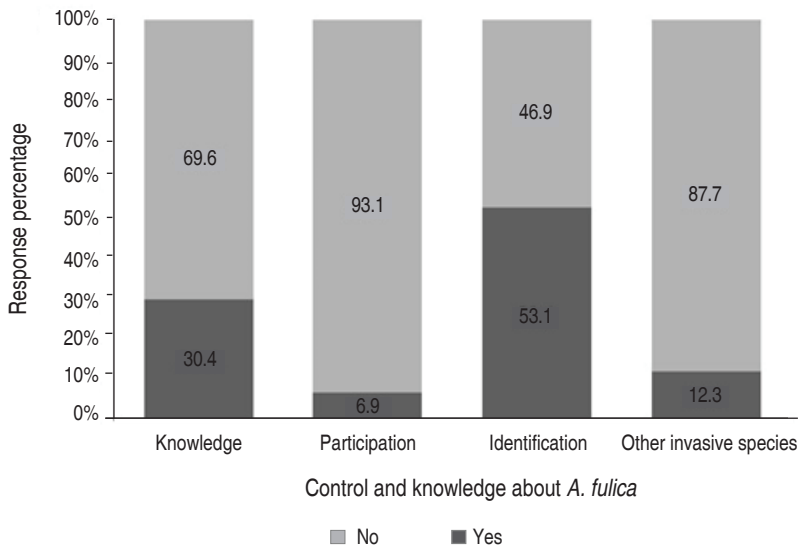

Figure 3. Perception of control efforts and knowledge on the giant African snail (*A. fulica*) by people interviewed in Cali's urban area (n=316).

The general knowledge perception of invasive species and methods of control were not related to any of the socio-demographic characteristics evaluated in this study. However, more men than women tended to associate this species with the negative impact of its presence on commerce, industry, and agriculture. People between 14 and 20 years old tended to react better to the presence of an invasive species around them (Table 2). It is not clear why people from Cali consider that the giant African snail is harmful in the context of public health if they do not know about the associated parasites. This association of the snail with public health could be attributed to the influence of mass media, which are the main source of divulgation on *Achatina fulica* in Colombia, although it is not the most reliable source (Russell and Balckburn, 2017; Patiño, 2018). Probably, the influence of the mass media on the people's knowledge about the giant African snail in the city of Cali is the reason why most of the variables of perception evaluated in this research depended on age but not on education level. Taking into account that the giant African snail was established as an invasive species in Colombia since 2008 (MAVDT, 2008) and that the public warning campaigns started since 2012, it is necessary to direct a more significant effort of sensitization and appropriation towards the citizens to get them involved in the management program as the key stakeholders.

There was a relationship of age, education level, and type of educative institution with the perception variables of giant African snails ($P<0.05$) (Table 3 and 4). People over 30 years of age had a negative perception about the level of knowledge on the mollusk ($P=0.001$), knowledge of parasites ($P=0.001$), other invasive species ($P=0.01$), sightings of the snail ($P=0.001$), knowledge of control ($P=0.004$), perception of the received information ($P=0.001$), and the work of the environmental authority

Table 2. Relationship between socio-demographic characteristics and perception variables on biological invasion.

Socio demographic character	Knowledge		Impact		Divulgation		Reaction		Report	
	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)	Yes (%)	No (%)
Gender										
Female	161(88)	21(12)	167(92)	15(8)	30(16)	152(84)	69(38)	113(62)	70(38)	112(62)
Male	124(93)	10(7)	130(97)	4(3)	29(22)	105(78)	53(40)	81(60)	60(45)	74(55)
P-value	0.256		0.058		0.247		0.815		0.298	
Age										
14-20	42(95)	2(5)	43(98)	1(2)	11(25)	33(75)	25(57)	19(43)	22(50)	22(50)
21-30	100(92)	9(8)	102(94)	7(6)	18(17)	91(83)	43(39)	66(61)	48(44)	61(56)
31-40	38(79)	10(21)	44(92)	4(8)	5(10)	43(90)	15(31)	33(69)	14(29)	34(71)
41-50	48(92)	4(8)	49(94)	3(6)	10(19)	42(81)	16(31)	36(69)	18(35)	34(65)
>50	56(90)	6(10)	59(95)	3(5)	15(24)	47(76)	23(37)	39(63)	27(44)	35(56)
P-value	0.119		0.801		0.287		0.076		0.222	
SE level[1]										
Low	76(90)	8(10)	79(94)	5(6)	20(24)	64(76)	36(43)	48(57)	38(45)	46(55)
Medium	159(88)	21(12)	170(94)	10(6)	30(17)	150(83)	66(37)	114(63)	68(38)	112(62)
High	50(96)	2(4)	48(92)	4(8)	9(17)	43(83)	20(38)	32(62)	24(46)	28(54)
P-value	0.247		0.805		0.376		0.627		0.385	
Educative level										
Elementary	7(100)	0(0)	7(100)	0(0)	4(57)	3(43)	3(43)	4(57)	5(71)	2(29)
High school	75(91)	7(9)	75(91)	7(9)	11(13)	71(87)	33(40)	49(60)	31(38)	51(62)
Technician	64(86)	10(14)	73(99)	1(1)	15(20)	59(80)	29(39)	45(61)	28(38)	46(62)
Undergraduate	83(90)	9(10)	83(90)	9(10)	16(17)	76(83)	34(37)	58(63)	37(40)	55(60)
Postgraduate	56(92)	5(8)	59(97)	2(3)	13(21)	48(79)	23(38)	38(62)	29(48)	32(52)
P-value	0.797		0.121		0.092		0.992		0.364	
School										
Public	136(89)	16(11)	145(95)	7(5)	30(20)	122(80)	62(41)	90(59)	57(38)	95(62)
Private	138(90)	15(10)	141(92)	12(8)	27(18)	126(82)	57(37)	96(63)	68(44)	85(56)
P-value	0.852		0.344		0.662		0.558		0.245	

[1] Socio-economic level.

(*P*=0.001), compared with people 14 to 29 years old. Deeper knowledge on this subject depends on the type of institution, with students or graduates from private schools being more aware of the issues surrounding them (*P*=0.038) (Table 3 and 4). Similar results have been reported in Brazil where a close relationship has been established between stratum and education level, with some perception variables similar to those evaluated in the present study (Sá *et al.,* 2016; Andreazzi *et al*., 2017; Shackleton *et al.,* 2018).

Table 3. Relationship between socio-demographic characteristics and perception variables on *A. fulica* in the city of Cali. 0 (low), 1 (medium), 3 (high).

Socio demographic characteristics	Level Knowledge			Ambiental damage			Health damage			Parasite Knowledge		Invasive species		Identification	
	0	1	2	0	1	2	0	1	2	Yes	No	Yes	No	Yes	No
								(%)							
Gender															
Female	59(33)	84(46)	38(21)	22(12)	58(32)	102(56)	16(8)	43(24)	123(68)	60(33)	122(67)	18(10)	163(90)	95(52)	86(48)
Male	40(30)	68(51)	26(19)	15(12)	42(31)	77(57)	15(11)	39(29)	80(60)	50(37)	84(63)	21(16)	113(84)	72(54)	62(46)
P-value		0.764			0.959			0.364			0.474		0.166		0.909
Age															
14-20	6(14)	27(61)	11(25)	1(2)	18(41)	25(57)	2(5)	16(36)	26(59)	24(55)	20(45)	8(18)	36(82)	36(82)	8(18)
21-30	27(25)	59(54)	23(21)	12(11)	40(37)	57(52)	7(6)	30(28)	72(66)	55(50)	54(50)	17(16)	91(84)	72(66)	37(34)
31-40	27(56)	16(33)	5(11)	9(19)	13(27)	26(54)	8(17)	12(25)	28(58)	8(17)	40(83)	0(0)	48(100)	18(38)	29(62)
41-50	20(38)	24(46)	8(16)	8(15)	13(25)	31(60)	8(15)	13(25)	31(60)	11(21)	41(79)	8(15)	44(85)	22(42)	30(58)
>50	19(31)	25(41)	17(28)	7(11)	16(26)	39(63)	6(9)	11(18)	45(73)	12(19)	50(81)	6(10)	56(90)	19(31)	43(69)
P-value		0.001			0.188			0.191			<0.001		0.01		<0.001
SE level[1]															
Low	36(43)	36(43)	12(14)	12(14)	24(29)	48(57)	8(10)	21(25)	55(65)	27(32)	57(68)	10(12)	74(88)	50(60)	34(40)
Medium	52(29)	86(48)	41(23)	22(12)	59(33)	99(55)	19(11)	47(26)	114(63)	68(38)	112(62)	20(11)	159(89)	97(54)	82(46)
High	11(21)	30(58)	11(21)	3(6)	17(33)	32(61)	4(8)	14(27)	34(65)	15(29)	37(71)	9(17)	43(83)	20(38)	32(62)
P-value		0.063			0.609			0.989			0.427		0.469		0.053
Educative level															
Elementary	2(29)	3(43)	2(29)	1(14)	4(57)	2(29)	0(0)	2(29)	5(71)	0(0)	7(100)	0(0)	7(100)	5(71)	2(29)
High school	25(30)	42(51)	15(18)	12(15)	27(33)	43(52)	11(13)	15(18)	56(68)	39(48)	43(52)	10(12)	72(88)	52(63)	30(37)
Technician	26(36)	30(41)	17(23)	11(15)	22(30)	41(55)	9(12)	22(30)	43(58)	20(27)	54(73)	9(12)	65(88)	35(47)	39(53)
Undergraduate	30(33)	47(51)	15(16)	8(9)	29(32)	55(60)	5(5)	27(29)	60(65)	33(36)	59(64)	12(13)	79(87)	51(56)	40(44)
Postgraduate	16(26)	30(49)	15(25)	5(8)	18(30)	38(62)	6(10)	16(26)	39(64)	18(30)	43(70)	8(13)	53(87)	24(39)	37(61)
P-value		0.818			0.604			0.483			0.014		0.992		0.033
School															
Public	53(35)	66(44)	32(21)	19(12)	48(32)	85(56)	14(9)	40(26)	98(64)	54(36)	98(64)	18(12)	133(88)	78(51)	74(49)
Private	43(28)	78(51)	32(21)	16(10)	48(31)	89(58)	16(10)	39(25)	98(64)	52(34)	101(66)	19(12)	134(88)	83(55)	69(45)
P-value		0.36			0.842			0.953			0.811		1		0.646

[1] Socio-economic level

Table 4. Relationship between socio-demographic characteristics and perception variables on control of *A. fulica* in the city of Cali. 0 (low), 1 (medium), 3 (high).

Socio demographic characteristics	Control Knowledge		Participation in control		Extern Knowledge		Satisfy with the information			Information reliability			Environmental authority		
	Yes	No	Yes	No	Yes	No	0	1	2	0	1	2	0	1	2
								(%)							
Gender															
Female	52(29)	130(71)	47(26)	135(74)	10(5)	172(95)	85(47)	74(41)	23(13)	73(40)	56(31)	53(29)	94(52)	74(41)	14(7)
Male	44(33)	90(67)	37(28)	97(72)	12(9)	122(91)	62(46)	46(34)	26(19)	46(35)	50(38)	37(28)	70(52)	52(39)	12(9)
P-value	0.458		0.267		0.797			0.212			0.421			0.883	
Age															
14-20	22(50)	22(50)	13(30)	31(70)	4(9)	40(91)	11(25)	20(45)	13(30)	7(16)	20(45)	17(39)	15(34)	23(52)	6(14)
21-30	39(36)	70(64)	35(32)	74(68)	12(11)	97(89)	34(31)	54(50)	21(19)	24(22)	42(39)	42(39)	49(45)	53(49)	7(6)
31-40	9(19)	39(81)	9(19)	39(81)	3(6)	45(94)	32(67)	13(27)	3(6)	29(60)	14(29)	5(10)	34(71)	14(29)	0(0)
41-50	12(23)	40(77)	13(25)	39(75)	2(4)	50(96)	38(73)	10(19)	4(8)	30(58)	11(21)	11(21)	33(63)	17(33)	2(4)
>50	14(23)	48(77)	13(21)	49(79)	1(2)	61(98)	32(52)	22(35)	8(13)	29(47)	18(29)	15(24)	32(52)	19(31)	11(18)
P-value	0.004		0.151		0.355			<0.001			<0.001			<0.001	
SE level[1]															
Low	24(29)	60(71)	27(32)	57(68)	5(6)	79(94)	45(54)	28(33)	11(13)	32(38)	32(38)	20(24)	37(44)	38(45)	9(11)
Medium	56(31)	124(69)	44(24)	136(76)	16(9)	164(91)	76(42)	71(39)	33(18)	67(37)	54(30)	58(32)	102(57)	64(36)	14(8)
High	16(31)	36(69)	13(25)	39(75)	1(2)	51(98)	26(50)	21(40)	5(10)	20(38)	20(38)	12(23)	25(48)	24(46)	3(6)
P-value	0.942		0.217		0.403			0.325			0.461			0.29	
Educative level															
Elementary	2(29)	5(71)	4(57)	3(43)	0(0)	7(100)	3(43)	3(43)	1(14)	2(29)	3(43)	2(29)	1(14)	2(29)	4(57)
High school	22(27)	60(73)	25(30)	57(70)	6(7)	76(93)	30(37)	34(41)	18(22)	25(30)	28(34)	29(35)	38(46)	35(43)	9(11)
Technician	22(30)	52(70)	15(20)	59(80)	3(4)	71(96)	41(55)	21(28)	12(16)	36(49)	23(32)	14(19)	47(64)	23(31)	4(5)
Undergraduate	31(34)	61(66)	24(26)	68(74)	10(11)	82(89)	40(43)	39(42)	13(14)	31(34)	32(35)	29(32)	49(53)	38(41)	5(5)
Postgraduate	19(31)	42(69)	16(26)	45(74)	3(5)	58(95)	33(54)	23(38)	5(8)	25(41)	20(34)	16(26)	29(48)	28(46)	4(7)
P-value	0.91		0.487		0.242			0.172			0.346			0.01	
School															
Public	49(32)	103(68)	32(21)	120(79)	9(6)	143(94)	76(50)	47(31)	29(19)	61(40)	48(32)	42(28)	72(47)	65(43)	15(10)
Private	44(29)	109(71)	49(32)	104(68)	12(8)	141(92)	66(43)	67(44)	20(13)	53(35)	54(35)	46(30)	84(55)	58(38)	11(7)
P-value	0.536		0.652		0.038			0.052			0.592			0.373	

[1] Socio-economic level

CONCLUSIONS

The people of the city of Cali of all economic conditions and all academic level, manifested a negative perception about the management actions carried out in the city by local environmental authority against the giant African snail invasion. This dissociation between people and the actions carried out by the local environmental authority must be solved to achieve the success of local management plan for this invasive species. Therefore, we recommend a deep adjustment of the institutional management policy that was established to respond as a city to the giant African snail invasion in Cali, being required that people become the central stakeholder.

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REFERENCES

Alcaldía de Santiago de Cali. 2018. Informe de gestión del municipio-2017. In: Alcaldía de Santiago de Cali, http://www.cali.gov.co/documentos/1166/informes-de-gestion-del-municipio/. 948 p.; accessed: March 2018.

Andreazzi MA, Gasparotto F, De Souza EA, Novais C, Cracco AF and Perez MA. 2017. Giant African snail, *Achatina fulica* (Férussac, 1821): an environmental and public health problem in the northwestern of Paraná State, Brazil. Acta Scientiarum. Biological Sciences 39(3): 301-311. doi: 10.4025/actascibiolsci.v39i3.35569

Balfour A and Ali N. 2014. An Economic Analysis of Three Management Options of the Giant African Snail (*Achtina fulica*) in Trinidad and Tobago. pp. 49-67. In: Ramnanan N (ed). Economic Impact of IAS in The Caribbean: Case Studies, CABI. Trinidad y Tobago. 88 p.

Castillo Herrera S and García Chacón GL. 2014. Población y control de caracol en el cultivar papaya en san antonio, Santa Rosa. Bachelor's Thesis in Agronomic Engineering. Facultad de Ciencias Agropecuarias. Universidad Técnica de Machala, Machala. 58 p.

Córdoba D, Patiño A and Giraldo A. 2017. Prevalence of *Strongylida* nematodes associated with African snail, *Achatina fulica*, in Valle del Cauca, Colombia. Revista MVZ Córdoba 22 (3): 6276-6286. doi: 10.21897/rmvz.1132

Correoso M. 2006. Estrategia preliminar para evaluar y erradicar *Achatina fulica* (Gastropoda: Achatinaceae) en Ecuador. Serie Zoológica 2: 45–52.

Crowley SL, Hinchliffe S and McDonald RA. 2017a. Conflict in invasive species management. Frontiers in Ecology and the Environment 15 (3): 133-141. doi: 10.1002/fee.1471

Crowley SL, Hinchliffe S and McDonald RA. 2017b. Invasive species management will benefit from social impact assessment. Journal of Applied Ecology 54(2): 351-357. doi: 10.1111/1365-2664.12817

da Silva JD and Marques G. 2017. *Achatina fulica* (BOWDICH, 1882) (Gastropoda-Stylommatophora-Achatinidae) medidas adotadas no controle da espécie em países da América do soul. Semioses 11(2): 1-7. doi: 10.15202/1981996x.2017v11n2p1

de La Ossa J, de La Ossa A, Castro J, Monroy M and Fuentes M. 2017. Incremento poblacional de *Achatina fulica* Bowdich 1822 (Mollusca: Gastropoda-Achatinidae) en una zona urbana de Sincelejo, Sucre, Colombia. Asociación Colombiana de Ciencias biológicas 1(29): 21-29.

Garcés MF, Patiño A, Gómez M, Giraldo A and Bolívar W. 2016. Sustancias alternativas para el control del caracol africano (*Achatina fulica*) en el Valle del Cauca, Colombia. Biota Colombiana 17(1): 44-52. doi: 10.21068/C2016v17r01a04

García M, Martín B, González JA, Alcorlo P and Montes C. 2008. Social perceptions of the impacts and benefits of invasive alien species: Implications for management. Biological Conservation 141(12): 2969-2983. doi: 10.1016/j.biocon.2008.09.003

Lowe S, Browne M, Boudjelas S and de Poorter M. 2000. 100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database. The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Comission (SSC) of the World Conservation Union (IUCN), Auckland. 12 p.

Luizaga L, Lima F and Barbosa L. 2015. Microbiological conditions and occurrence of the African land snail in Araguaína city at Tocantins State, Brazil / Condições microbiológicas e de ocorrência do caramujo terrestre africano na cidade de Araguaína no Estado do Tocantins. Journal of Bioenergy and Food Science 2(4): 234-238. doi: 10.18067/jbfs.v2i4.62

Ministerio de Ambiente, Vivienda y Desarrollo Sostenible-MAVDT. 2008. Resolución 0848 de 2008. Colombia.

Ministerio de Ambiente, Vivienda y Desarrollo Sostenible-MAVDT. 2011. Resolución 654 de 2011. Colombia.

Moreira Z, Souza S and Carvalho S. 2012. O caramujo africano em contexto escolar: as percepções de estudantes do ensino fundamental e o estudo da helmintofauna associada ao caramujo em Barra do Piraí (RJ). Ensaio Pesquisa em Educação em Ciências Supl. 1. 14(3): 275-288.

Patiño A and Giraldo A. 2017. Variación génica intrapoblacional del caracol africano (*Achatina fulica*) en el d Valle del Cauca. Revista MVZ Córdoba: 5925-5937. doi: 10.21897/rmvz.1028

Patiño. A. 2018. Caracol africano en el Valle del Cauca: Estado actual de la información, Morfología y Control alternativo. Master's Thesis in Science-Biology. Facultad de Ciencias Naturales y Exactas. Universidad del Valle. Cali. 80 p.

Peng J, He ZP, Zhang S, Lun ZR, Wu ZD, Fan CK, Brown CL, Cheng PC, Peng SY and Yang TB. 2017. Phylogeography of *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae) in southern China and some surrounding areas. PLoS Neglected Tropical Diseases 11(8). doi: 10.1371/journal.pntd.0005776

Pereyra PJ. 2016. Revisiting the use of the invasive species concept: An empirical approach. Austral Ecology 41(5): 519-528. doi: 10.1111/aec.12340

Raut SK and Barker GM. 2002. Chapter 3: *Achatina fulica* Bowdich and other Achatinidae as Pests in Tropical Agriculture. pp.

55-114. In: Baker GM (ed.) Molluscs as crop pests. CABI Publishing. Wallingford, Reino Unido. 468 p.

Roda A, Nachman G, Weihman S, Cong MY and Zimmerman F. 2016. Reproductive ecology of the giant African snail in South Florida: Implications for Eradication Programs. PLoS ONE 11(11). doi: 10.1371/journal.pone.0165408

Rout TM, Moore JL and McCarthy MA. 2014. Prevent, search or destroy? A partially observable model for invasive species management. Journal of Applied Ecology 51(3): 804-813. doi: 10.1111/1365-2664.12234

Russell JC and Blackburn TM. 2017. The rise of invasive species denialism. Trends in Ecology & Evolution 32(1): 3-6. doi: 10.1016/j.tree.2016.10.012

Sá JC, Araújo FL, Teixeira RG, Dos Santos WS and Ferrari SF. 2016. Education as controlling factor of invasive species (*Achatina fulica*) in an amazonian city, Brazil. Creative Education 7(1): 159-170. doi: 10.4236/ce.2016.71016

Shackleton RT, Adriaens T, Brundu G, Dehnen-Schmutz K, Estévez RA, Fried J, Larson BMH, Liu S, Marchante E, Marchante H, Moshobane MC, Novoa A, Reed M and Richardson DM. 2018. Stakeholder engagement in the study and management of invasive alien species. Journal of Environmental Management. 229: 88-101. doi: 10.1016/j.jenvman.2018.04.044

Smith TR, White-Mclean J, Dickens K, Howe AC and Fox A. 2013. Efficacy of four molluscicides against the giant African snail, *Lissachatina fulica* (Gastropoda: Pulmonata: Achatinidae). Florida Entomologist 96(2): 396–402. doi: 10.1653/024.096.0202

Thiengo SC, Faraco FA, Salgado NC, Cowie RH and Fernandez MA. 2007. Rapid spread of an invasive snail in South America: the giant African snail, *Achatina fulica* in Brasil. Biological Invasions 9(6): 693-702. doi: 10.1007/s10530-006-9069-6

Varela RE, Arias JS and Velásquez LE. 2017. Estandarización de una prueba de reacción en cadena de la polimerasa en tiempo real múltiple para la identificación de *Angiostrongylus cantonensis*, *A. costaricensis* y *A. vasorum*. Biomédica 38(1): 111-119 doi: 10.7705/biomedica.v38i0.3407

Waliczek TM, Williamson PS and Oxley FM. 2017. College student knowledge and perceptions of invasive species. HortTechnology 27(4): 550-556. doi: 10.21273/HORTTECH03709-17

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Fuzzy system to evaluate performance and the physiological responses of piglets raised in the farrowing house with different solar heating systems

Sistema fuzzy para evaluar el rendimiento y las respuestas fisiológicas de las lechones en la casa de granjas con diferentes sistemas de calefacción solar

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ABSTRACT

The present work aims to develop a mathematical model, based on fuzzy set theory, for predicting performance and the physiological responses of piglets raised in the farrowing house with different solar heating systems. To do this, a solar heater prototype was constructed using alternative materials and the heating efficiency was compared with a commercial solar heater system. In order to thermally evaluate the heaters, temperature sensors were installed in the inlet and outlet pipes of each floor and thermal reservoir. The fuzzy system was developed and the variables dry air bulb temperature (T_{bs}) and relative humidity (RH) of the air were defined as inputs. Based on the input variables, the fuzzy system predicts the productive performance (weight gain - WG) and physiological responses (respiratory rate - RR, rectal temperature - RT, and skin temperature - ST) of piglets raised in an environment with solar heating. Based on the results, the fuzzy model was adequate for predicting the physiological responses and productive performance of piglets, presenting low standard deviation and high correlation with the validation data. This model can be used to assist producers in decision making, especially regarding maintaining animal welfare while the thermal environment changes.

Keywords:
Computational simulation
Swine
Thermal comfort

RESUMEN

El presente trabajo tiene como objetivo desarrollar un modelo matemático, basado en la teoría de conjuntos difusos, para predecir el rendimiento y las respuestas fisiológicas de lechones criados en la casa de maternidad con diferentes sistemas de calefacción solar. Para ello, se construyó un prototipo de calentador solar utilizando materiales alternativos y se comparó la eficiencia de calentamiento con un sistema de calentador solar comercial. Con el fin de evaluar térmicamente los calentadores, se instalaron sensores de temperatura en las tuberías de entrada y salida de cada piso y depósito térmico. El sistema difuso se desarrolló y las variables temperatura de bulbo de aire seco (T_{bs}) y humedad relativa (HR) del aire se definieron como entradas. Con base en las variables de entrada, el sistema difuso predice el rendimiento productivo (ganancia de peso - WG) y las respuestas fisiológicas (frecuencia respiratoria - RR, temperatura rectal - TR y temperatura de la piel - ST) de los lechones criados en un ambiente con calentamiento solar. Con base en los resultados, el modelo difuso fue adecuado para predecir las respuestas fisiológicas y el rendimiento productivo de los lechones, presentando baja desviación estándar y alta correlación con los datos de validación. Este modelo puede utilizarse para ayudar a los productores en la toma de decisiones, especialmente en lo que respecta al mantenimiento del bienestar animal mientras el entorno térmico cambia.

Palabras clave:
Simulación computacional
Porcinos
Confort térmico

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Pig farming is an activity that requires a lot of dedication from the breeder to achieve good productivity levels and, consequently, satisfactory economic results. Indoor air temperature, humidity, and air quality in rearing environment are important factors that can affect the health, productivity, and welfare of livestock and poultry in confined animal feeding operations (Carroll *et al*., 2012; Kim *et al*., 2008).

Air temperature is a key environmental factor for pigs because pigs are warm-blooded animals and a constant body temperature is the basis of their normal life (Huynh *et al*., 2005). Humidity is another important environmental factor that affects pig body heat-regulation. Humidity generally has minor effects on pigs. However, when combined with high ambient temperature, a significant difference in average daily weight gain in pigs was found (Xie *et al*., 2017).

In the case of the farrowing house, this problem is evidenced by the coexistence within it of two categories with very different environmental requirements. On the one hand is the sow which must be cooled, and, on the other hand are the piglets, which must be heated. The range of thermal comfort in the environment for piglets during the first days of life is between 32 and 34 °C and that for the sow is in the range 16 to 21 °C (Perdomo *et al*., 1987). The solution of this problem, present in all pig farms, is a priority when seeking to improve the performance of both categories. Thus, within the principles of thermal comfort and animal welfare, the producer is faced with a major problem, where in a small physical space it is required to provide two different microenvironments where otherwise the performance of both the sows and the piglets will be compromised (Pandorfi *et al*., 2007).

In general, the heating of piglets in maternity phase is a system that consumes a lot of power on the farm. Thus, because of the large amount of electricity that is used in this type of setting, there is a need for further research to enable minimal consumption without harming animal welfare while preserving the environment.

Success in intensive livestock production is directly related to the efficient management of the environment. The control of the housing environment is generally based on measurements of temperature and relative humidity. However, surveys indicate the potential for more accuracy in decisions when physiological responses of animals to environmental stressors are incorporated (Goedseels *et al*., 1992; Aerts *et al*., 1996; Lacey *et al*., 2000).

The difficulty of analyzing the large volume of information relating to all variables involved in the establishment of appropriate conditions for animals in the building has been reported in the literature. The main interest in this area is related to the study of phenomena that exhibit gradual uncertainties and can be modeled by Fuzzy Set Theory. Because of its explanatory and multi-disciplinary power this theory can facilitate the work of the modeler in incorporating specialist knowledge of the area, improving the analysis and understanding of some real situations (Amendola *et al*., 2004).

Fuzzy methodology has been used in various fields such as data analysis, expert systems, control and optimization, aircraft control and biomedicine (Ribacionka, 1999; Lopes, 1999; Weber and Klein, 2003). In the animal ambient area, various applications indicate its potential use, such as in the study of thermal comfort in poultry (Gates *et al*., 2001; Amendola *et al*., 2004; Yanagi *et al*., 2006) and pigs (Xie *et al*., 2017; Queirós and Nääs, 2005), besides being used to detect estrus in dairy cows (Firk *et al*., 2003; Ferreira *et al*., 2007a).

The fuzzy logic application in the pig farrowing house environment becomes necessary and valid to quantify thermal comfort of piglets and at the same time, the productive performance and physiologic responses of these animals. Thus, this method becomes a reliable tool in the predetermination of the comfort of maternity pigs, helping to reduce errors and increase the productivity and welfare of these animals.

Thus, in view of the enormous importance of the production chain of pigs in Brazil, hence the imperative need to seek more sustainable solutions that ensure the minimization of the impact of this activity on the environment, this paper aims to develop a mathematical model based on fuzzy set theory to predict the production

performance and the physiological responses of piglets raised in a farrowing house with different heating systems and to validate that model using field data.

MATERIALS AND METHODS

Characterization of the farrowing house and installation of heating systems

The entire study was conducted during the 2015 summer period, in a pig farrowing house of the experimental center in pig farming at the Federal University of Lavras, in Lavras - MG, Brazil. The city of Lavras is located at 21°14' latitude south, 40°00' longitude west of Greenwich. The climate, according to the Köppen climate classification is Cwa, rainy temperate (mesothermal) with dry winter and rainy, subtropical summer, altitude of 918.84 m with an average temperature of 19.4 °C, annual average precipitation 1529.7 mm and 76.2% relative humidity.

The farrowing house used in this experiment had the following design characteristics: dimensions of 8.26 m wide and 8.40 m long, ceiling height of 2.15 m, gable roof, wood structure, covered with ceramic tiles and East-West orientation. The farrowing house floor was compact concrete with a slope of 2.0% towards the gutters. Five pens 1.80 m long by 1.35 m wide were installed within them, connected to the wooden creep feeders 1.00 m long by 0.68 m wide (Figure 1A), with a shield against the crushing of piglets. Each pen had a perforated floor of durable plastic. A fan/nebulizer was installed inside the farrowing house.

In the pig farrowing house evaluated in this study, two different heating systems were installed: a) creep feeder equipped with heated concrete floor, with hot water pipe heating through solar water heater built with alternative materials (ASWH); b) creep feeder equipped with thermal concrete floor heated through hot water pipes by a solar water heater built with conventional materials (CSWH). Distributions of heating systems can be seen by the diagram in Figure 1B.

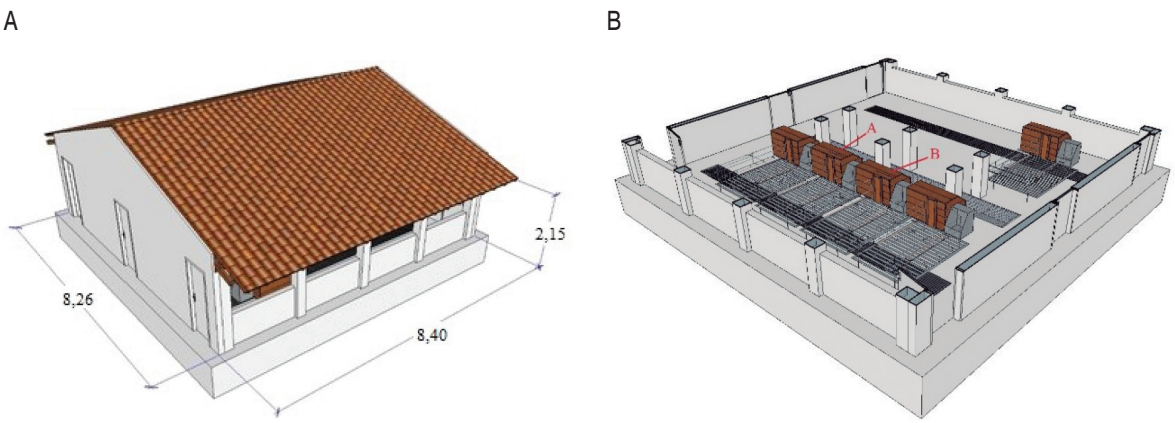

Figure 1. Schematic drawings. A. Of farrowing house with the main dimensions, in meters; B. Of the distribution of the different heating systems within the farrowing house. Caption: A – ASWH and B – CSWH.

The conventional solar water heating system (CSWH) had a glass plated solar collector made of aluminum, with internal fins painted in matte black to absorb sunlight and transfer energy to internal piping. The components of the thermal reservoir had internal cylinders and pipes manufactured with stainless steel and rigid expanded polyurethane.

The prototype of solar water heaters manufactured with alternative materials (ASWH) was built with PVC pipes and connections (1/2" diameter), PET bottles and milk cartons (Tetra Pak®). The milk cartons were painted matte black to absorb heat, and retain it within the cylinders to be transferred to water through the PVC pipes which were also painted matte black. In the construction of the alternative solar heater prototype 60 bottles of transparent polyethylene terephthalate (PET) of 2 liters were used. The milk cartons were opened on the upper and lower part, where all the boxes were cut using a cutting jig proposed by CELESC (2010) to maintain a standard in all cases.

In an alternative construction of the hot water reservoir a fiberglass water box of 50 liters was used, coated with polystyrene plates (30 mm), duct tape, and self-adhesive asphalt and aluminum blanket (2.5 mm) to protect the polystyrene plates from the weather. Four 20 mm holes were made in the reservoir, with two holes for circulation of water between the thermal reservoir and the solar collector and the other two holes for water circulation from the heat reservoir to the floor.

In the electrical resistance heater system heater cables were distributed within a masonry floor, and the temperature was controlled by means of an analog thermostat (100 W).

To test the two water heating systems two masonry floors were built with dimensions 74 cm long, 46 cm wide and 7 cm thick. To reduce the heat dissipation at the base of the floors, we used 30 mm polystyrene plates. A 20 mm galvanized steel pipe was placed in the two floors, forming a coil, aiming to uniformly distribute the heat from the water inside the floor. In the third floor heating cables were installed controlled by means of an analog thermostat.

The two solar collectors and water tanks were placed at a distance of about 10 m from the farrowing house to avoid shading them (Figure 2). A low flow water pump (mod. ZC-T40, 12V and 1.05A) was used in each system to force the circulation of water within each system. On each floor a digital controller (thermostat) was used, designed for solar heating applications, which operated to control water flow through the temperature differential between the floor entrance and the thermal reservoir.

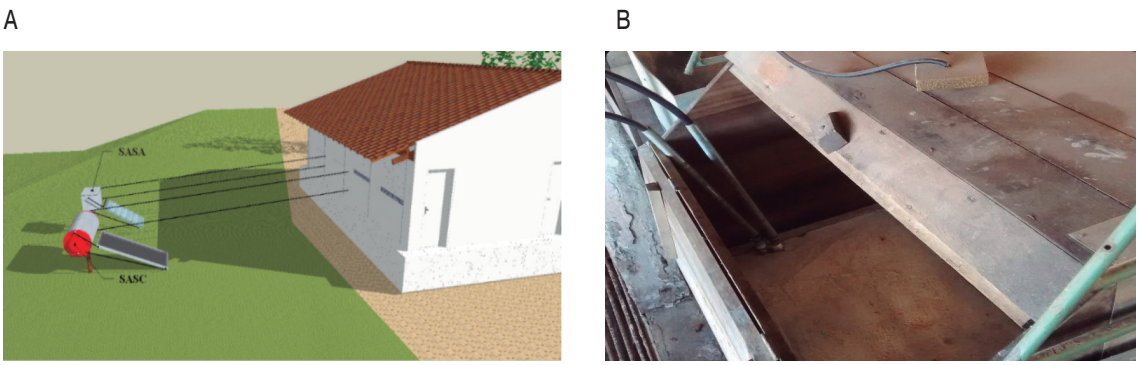

Figure 2. A. Functional diagram of the thermal floor and B. Detail of the concrete floor inside the creep feeder.

The pigs used in this study were from sows of the same order of delivery and were equalized in order to eliminate interference factors, maternal ability, number of piglets/litter, etc. Each pen presented between 8 and 12 piglets that, after birth, were relocated by criteria of weight and number of animals so that a fixed number of 10 piglets remained in all the feeders studied.

Measurement and Instrumentation

During all stages of the study, the environmental variables were monitored in the creep feeder, in the environment internal to and external to the farrowing house, through sensors/recorder systems with automated registration. The following variables make up the thermal environment: dry bulb air temperature (T_{bs}), relative humidity (RH) and air velocity (V_{air}).

The registration of these environmental variables was performed every 10 minutes for 24 hours daily for the first 21 days of life of the piglets, at a point allocated within each creep feeder.

Sensors/registers of T_{bs} and RH (Hobo® Mod. U12-012 and accuracy of ±2.5%) were housed inside a perforated guard container, to prevent damage to equipment caused by piglets or excess moisture, and, therefore, the readings were compared to another external sensor outside the protection, to verify any interference of the protection on the reading equipment. The air velocity (V_{air}) is manually measured by means of a hot-wire anemometer (Testo® Mod. 416 and resolution of 0.1 m s^{-1}). In creep feeders, sensors/registers were fixed in the cover of the creep feeders at a distance of approximately 0.5 m from the

floor. In the farrowing house, the environmental variables were recorded within the facility, in the center of the pens that will be studied at a height of 1.3 m from the floor, approximately. In the area outside the premises, sensors/recorders were installed inside a weather shelter, height 1.5 m from the surface, which represented the microclimate of the site.

Physiological responses were evaluated by rectal temperature (RT), skin temperature (ST) and respiratory rate (RR). The RT and RR were measured by two examiners. The RT was obtained with the aid of a digital clinical thermometer (Brasmed® and precision of ±1.0 °C) inserted approximately 2.5 cm into the rectum of animals. A gel-alcohol solution (70%) was used on the tip for asepsis of the digital clinical thermometer. RR was determined from direct visual observation for 15 seconds and then extrapolated for 1 minute. The ST was recorded using a thermographic camera (Instrutemp®, mod. ITTMV-100, accuracy of ±2% of the reading). Thermographic images were obtained covering the entire length of the animal (head, back, lower back and leg). ST was recorded during the morning (9:00 to 11:00) and afternoon (15:00 to 17:00) daily during the first four weeks of the animals' lives. The images were processed using the software of the camera itself, from points randomly selected. The emissivity of 0.95 was adopted (Montanholi *et al.*, 2008).

The productive performance was assessed by daily weight gain (WG) of the animals. For the determination of WG, five animals were randomly selected and weighed used a digital scale.

Fuzzy model

The fuzzy model developed aimed to generate a system for decision-making on the thermal comfort and productive performance and physiological responses of piglets raised in a farrowing house with a solar heating system. To make this possible, several parameters were evaluated carefully, since the fuzzy system depends on a robust knowledge base to meet the results expected by the user.

To develop the fuzzy system input variables were defined as: the dry bulb air temperature (T_{bs}) and relative humidity (RH), obtained during the data collection in the environment within the creep feeders. Based on the input variables, the fuzzy system predicts growth performance (weight gain, WG) and physiological responses (respiratory rate - RR, rectal temperature - RT and skin temperature - ST) of piglets raised in farrowing house with solar heating systems.

144 data sets were selected from the data collected in the field experiment for the two different solar heating systems evaluated, where the thermal comfort characteristic behavior was directly influenced by T_{bs} and RH. Of this total, 33.4% (48 data sets) were used in the development of the relevance functions and rules and 66.6% (96 data sets) were used to test the model developed.

The model was developed in MATLAB® 7.1 through the fuzzy toolbox. The inference method used in the analysis was the Mamdani method, which combines the degrees of relevance for each of the input values by the minimum operator, and adds the rules with the maximum operator (Leite *et al.*, 2010) and is used by various authors (Nascimento *et al.*, 2011; Yanagi *et al.*, 2012; Tavares and Schiassi, 2016). The defuzzification is performed by the center of gravity method. The fuzzy rule system was created based on the database obtained experimentally, on information from the literature and with the help of experts.

Three experts were selected according to the expert fuzzy selection methodology proposed by Cornelissen *et al.* (2003) and used by several authors (Yanagi *et al.*, 2012; Schiassi *et al.*, 2012). This feature is desired by a specialist (Ayyub and Klir 2006), given its direct influence on reliability and quality of results (Martino, 1993; Preble, 1984).

The rule system (Table 1) was developed based on combinations of T_{bs} and RH, and an expert was consulted to prepare the output result for each combination of the input data. All ratings were established according to the boundary conditions mentioned in the works of Esmay (1982); Nääs *et al.* (1998).

From the relationship between the input variables, a fuzzy model using the Mandani method has been formulated, trapezoidal functions having been selected for input variables (Figure 3), according to Esmay (1982), Nääs (1989); Nääs *et al.* (1998).

Table 1. Fuzzy sets for the input variables.

Variables	Fuzzy sets			
	1	2	3	4
T_{bs} (°C)	[20; 25]	[25; 32]	[32; 40]	–
RH (%)	[0; 40]	[40; 70]	[70; 90]	[90; 100]

For output variables (RR, ST, RT and WG), the values were grouped according to the characteristic behavioral patterns related to growth performance and physiological responses. These values were measured with the aid of two experts who were consulted to produce the output result for each combination of input data (Table 2).

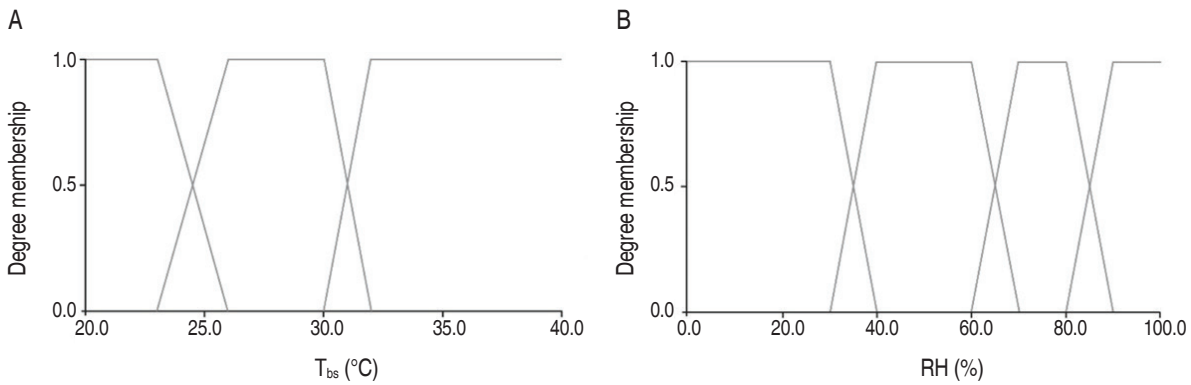

Figure 3. Relevance functions of the fuzzy sets accepted by input variables: A. T_{bs}, in °C; B. RH, in %.

Table 2. Range of fuzzy sets for output variables.

Intervals	Fuzzy sets			
	RR	RT	ST	WG
1	[50.0; 56.8]	[38.1; 38.6]	[32.5; 33.3]	[1.7; 2.3]
2	[52.8; 60.6]	[38.6; 39.1]	[33.1; 34.9]	[2.1; 2.5]
3	[58.6; 74.6]	[39.0; 39.2]	[34.8; 35.2]	[2.4; 2.7]
4	[72.6; 84.5]	[39.2; 39.5]	[35.0; 35.4]	[2.6; 3.0]
5	[81.4; 88.2]	[39.4; 39.7]	[35.3; 36.0]	[2.9; 3.4]
6	[86.9; 97.5]	[39.6; 40.3]	[35.7; 37.0]	[3.3; 3.8]
7	[92.8; 113.0]	[40.1; 40.5]	–	[3.6; 4.3]
8	–	–	–	[4.1; 4.6]
9	–	–	–	[4.5; 4.7]

After preliminary testing settings, the triangular model was used for the output variable relevance functions (Figure 4). In accordance with combinations of input data 12 rules are defined, as can be seen in Table 3.

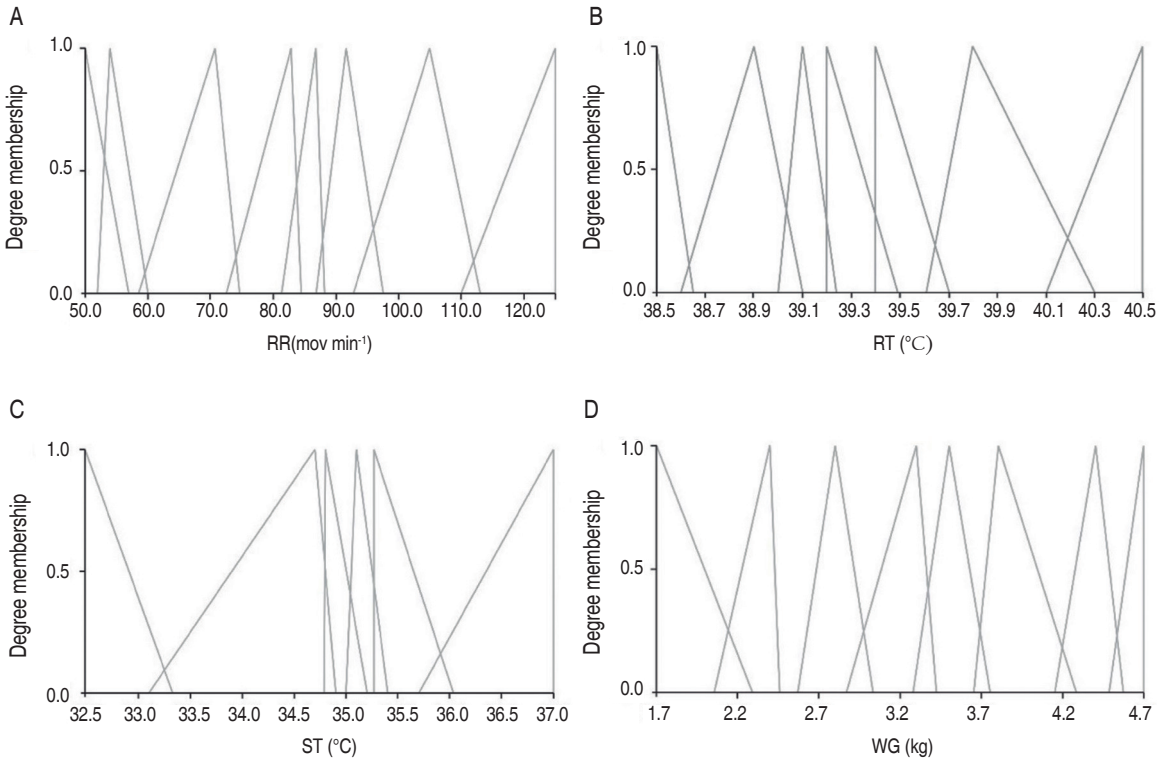

Figure 4. Relevance functions of accepted fuzzy sets according to the output variables: A. RR, in mov.min^{-1}; B. RT, in °C; C. ST, in °C; D. WG, in kg.

Table 3. System of fuzzy inference rules for relative humidity (RH), dry bulb air temperature (T_{bs}).

	Rules
1	If (RH = 1) and (T_{bs} = 1), then (RR = 1), (RT = 1), (ST = 4) and (WG = 2);
2	If (RH = 1) and (T_{bs} = 2), then (RR = 3), (RT = 3), (ST = 3) and (WG = 3);
3	If (RH = 1) and (T_{bs} = 3), then (RR = 6), (RT = 4), (ST = 4) and (WG = 2);
4	If (RH = 2) and (T_{bs} = 1), then (RR = 2), (RT = 5), (ST = 3) and (WG = 9);
5	If (RH = 2) and (T_{bs} = 2), then (RR = 3), (RT = 2), (ST = 6) and (WG = 4);
6	If (RH = 2) and (T_{bs} = 3), then (RR = 4), (RT = 5), (ST = 2) and (WG = 1);
7	If (RH = 3) and (T_{bs} = 1), then (RR = 5), (RT = 7), (ST = 5) and (WG = 5);
8	If (RH = 3) and (T_{bs} = 2), then (RR = 5), (RT = 4), (ST = 1) and (WG = 3);
9	If (RH = 3) and (T_{bs} = 3), then (RR = 7), (RT = 6), (ST = 5) and (WG = 6);
10	If (RH = 4) and (T_{bs} = 1), then (RR = 1), (RT = 3), (ST = 4) and (WG = 7);
11	If (RH = 4) and (T_{bs} = 2), then (RR = 3), (RT = 3), (ST = 4) and (WG = 8);
12	If (RH = 4) and (T_{bs} = 3), then (RR = 8), (RT = 1), (ST = 5) and (WG = 6).

RESULTS AND DISCUSSION

The fitting of the fuzzy model was performed based on the interval data collected during the experiment with the range for each relevance function of the output variables adopted in order to result in the lowest possible error when compared with experimentally measured data.

Thus, to test the fuzzy model, measured data of T_{bs} and RH for the first three weeks of life of pigs were used while model output results were compared with RF, RT, ST and WG values obtained through an experiment conducted in the swine farrowing house with the solar heating systems (Tables 4 and 5).

Table 4. Comparison of the respiratory rate values (RR, mov min^{-1}), Rectal Temperature (RT, °C) obtained experimentally (ME) and simulated (MF) by the fuzzy model.

	Input		ME		MF		SD		Error (%)	
			RR	RT	RR	RT	RR	RT	RR	RT
1	RH1	$T_{bs}1$	52.0	38.5	52.0	38.5	0.00	0.01	0.00	0.05
2	RH1	$T_{bs}2$	68.0	39.1	68.0	39.1	0.00	0.03	0.00	0.10
3	RH1	$T_{bs}3$	92.0	39.3	92.0	39.3	0.00	0.01	0.00	0.05
4	RH2	$T_{bs}1$	56.0	39.5	56.0	39.5	0.00	0.01	0.00	0.05
5	RH2	$T_{bs}2$	68.0	38.9	68.0	38.9	0.00	0.02	0.00	0.08
6	RH2	$T_{bs}3$	80.0	39.6	80.0	39.5	0.00	0.07	0.00	0.25
7	RH3	$T_{bs}1$	84.0	40.2	85.5	40.4	1.06	0.15	1.79	0.52
8	RH3	$T_{bs}2$	86.0	39.2	85.5	39.3	0.35	0.08	0.58	0.29
9	RH3	$T_{bs}3$	104.0	39.8	104.0	39.9	0.00	0.10	0.00	0.35
10	RH4	$T_{bs}1$	52.0	39.1	52.0	39.1	0.00	0.02	0.00	0.08
11	RH4	$T_{bs}2$	68.0	39.1	68.0	39.1	0.00	0.01	0.00	0.05
12	RH4	$T_{bs}3$	120.0	38.6	120.0	38.5	0.00	0.07	0.00	0.26
	Average						0.12	0.05	0.20	0.18

Table 5. Comparison of the skin temperature (ST, °C) and weight gain (WG, kg) obtained experimentally (ME) and simulated (MF) by the fuzzy model.

	Input		ME		MF		SD		Error (%)	
			ST	WG	ST	WG	ST	WG	ST	WG
1	RH1	$T_{bs}1$	35.1	2.3	35.1	2.31	0.00	0.02	0.00	1.09
2	RH1	$T_{bs}2$	34.7	2.5	34.8	2.51	0.07	0.00	0.29	0.00
3	RH1	$T_{bs}3$	35.2	2.3	35.2	2.31	0.00	0.02	0.00	1.07
4	RH2	$T_{bs}1$	34.8	4.6	34.8	4.64	0.00	0.06	0.00	1.98
5	RH2	$T_{bs}2$	36.8	2.8	36.6	2.80	0.14	0.01	0.54	0.54
6	RH2	$T_{bs}3$	34.5	2.0	34.3	1.89	0.14	0.10	0.58	7.13
7	RH3	$T_{bs}1$	35.5	3.3	35.5	3.20	0.00	0.09	0.00	3.90
8	RH3	$T_{bs}2$	32.7	2.6	32.8	2.51	0.10	0.07	0.43	4.05
9	RH3	$T_{bs}3$	35.3	3.5	35.5	3.52	0.14	0.00	0.57	0.14
10	RH4	$T_{bs}1$	35.1	3.8	35.1	3.91	0.00	0.05	0.00	1.96
11	RH4	$T_{bs}2$	35.2	4.4	35.1	4.38	0.07	0.00	0.28	0.11
12	RH4	$T_{bs}3$	35.4	3.4	35.5	3.52	0.07	0.06	0.28	2.05
	Average						0.06	0.04	0.25	2.05

According to Pandorfi (2005), the ambient temperature range for comfort during the first week of life of the piglets, is between 32 to 34 °C. In this study, the maximum WG and the best RR, RT and ST were achieved at slightly lower conditions of 32 °C (Tables 4 and 5). Lower values of WG and worse of RR, RT and ST were found with T_{bs} and RH above 32°C and 70%.

Queirós and Nääs (2005) developed an ideal standard of environmental comfort for pigs in the nursery using the fuzzy methodology. Thus, the authors concluded that the ideal standard for pig production in the nursery lies in T_{bs} of 29 °C and RH of 75%.

Low temperatures in the farrowing house can cause stressful conditions for piglets. The reduction in T_{bs} in the environment of piglets below 20 °C can result in decrease of consumption of colostrum and of rectal temperature and therefore generate an increase in heat generation leading to mobilization of body reserves (Dividich and Noblet, 1981). In this study, the heating systems tested had a T_{bs} above 20 °C (Table 5).

Piglets after birth suffer from a sudden drop in ambient temperature, with a reduction from 1.7 to 6.7 °C in body temperature (Pandorfi, 2005), causing neonatal hypothermia. Under these conditions, the pigs reduce their motor activity and consequently reduce colostrum intake, causing increased incidence of diseases, more crushed piglets and a high rate of rejects at weaning, requiring some special care (Carvalho *et al.*, 2006; Carvalho *et al.*, 2013). Turco *et al.* (1998) mentions that a suitable environment for lactating sows may facilitate the production of milk and, consequently, allow increased weight gain of the piglets.

The mean standard deviations of the variables RR, RT, ST and WG were 0.12 mov min^{-1} 0.05 °C, 0.06 °C and 0.04 kg, respectively, corresponding to the measured percentage error of 0.20, 0.18, 0.25 and 2.05%, as can be seen in Tables 4 and 5.

Analyzing the physiological responses and productive performance of piglets due to the different values of T_{bs} and RH evaluated, there is wide variation in the experimentally measured data at the same time that the fuzzy model results developed are suited to these variations, making the precision of this system clear for adapting to different conditions and combinations of database values used in the testing of this model.

In order to test the accuracy of the proposed model, linear regressions were carried out and the results showed coefficient of determination (R^2) equal to 0.999, 0.980 and 0.984 for RR, RT and ST, respectively (Figure 5). These results indicate good accuracy for the fuzzy model evaluated in this study. The operationalization of these results supports the control decision for the heating system in creep feeders, thus ensuring better production.

Medeiros *et al.* (2014), in creating mathematical models to estimate CR, WG and CA in adult chickens as a function of T_{bs} RH and air velocity (V_{air}), found values of R^2 equal to 0.91, 0.89 and 0.72, respectively.

When comparing the WG values simulated by the fuzzy model with those obtained experimentally in maternity pigs in the study (Tables 4 and 5), it was found that the standard deviation of values, percentage error and coefficient of determination (R^2) were 0.04 g, 2.05% and 0.994, respectively. These results indicate that the fuzzy model proposed had adequate precision for the prediction of WG in piglets.

Xie *et al.* (2017) prediction model of NH_3 emission from a fattening pig room based on the indoor concentration using adaptive neuro fuzzy inference system observed that percentage error and coefficient of determination (R^2) between output and testing data were 0.0436 and 63.5%.

According to Castro *et al.* (2013), considering that variations in WG would involve making a decision, the fuzzy system could be triggered to emit a warning signal, thus preventing the exposure of piglets to a harmful environment, reducing the heat stress on animals and possible production losses.

Fernandes *et al.* (2011) in evaluating the physiological behavior and performance indexes of the sows and piglets with the use of floor heating and cooling systems, mention that the heating system provided piglets at the end of the study with a WG increase of 28% (4.890 kg) compared to the control (3.800 kg).

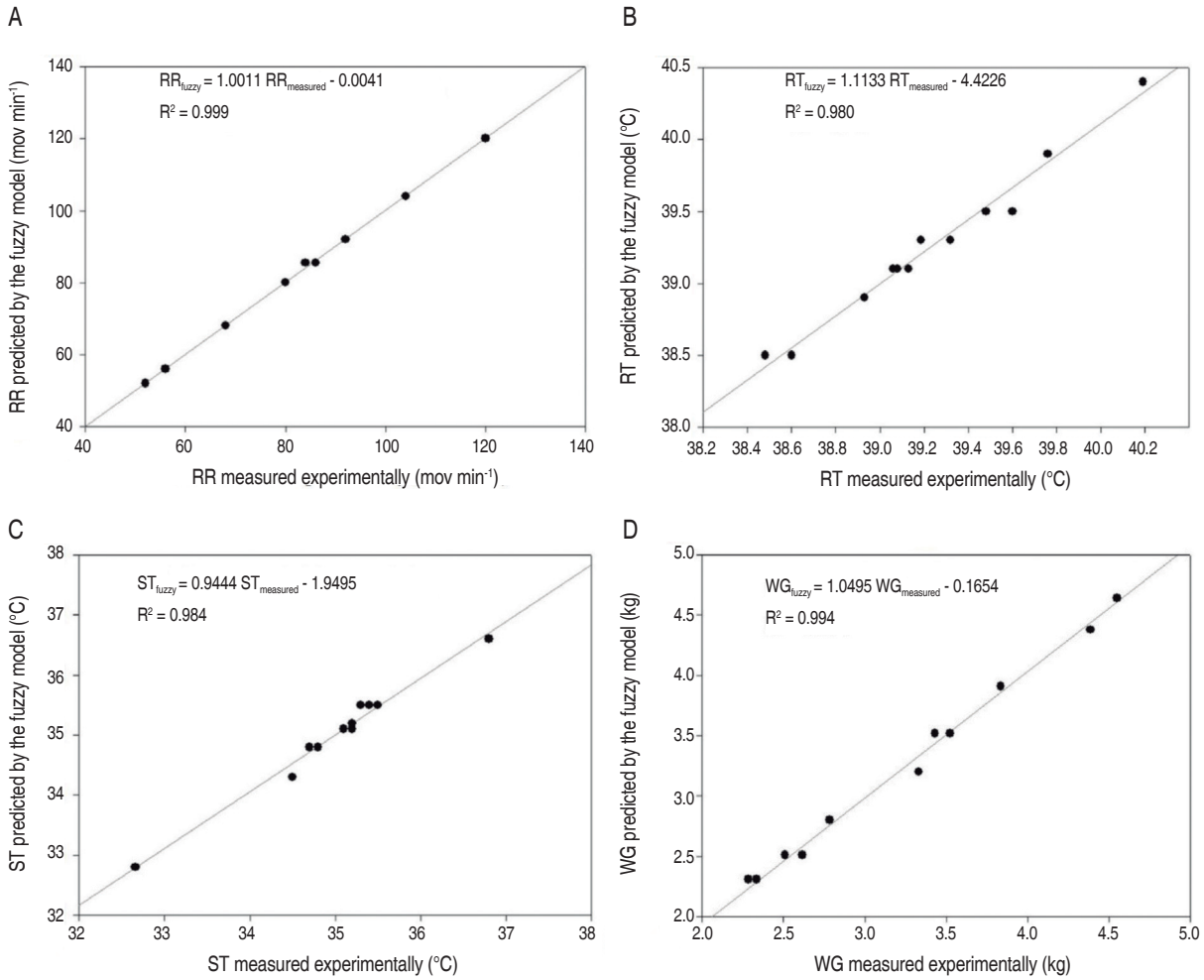

Figure 5. Linear regressions for the output variables: A. Respiratory rate (RR); B. Rectal temperature (RT); C. Skin temperature (ST); D. Weight gain (WG), depending on the values predicted by the fuzzy model and the values measured experimentally.

The improved weight gain in breast-feeding will present a positive effect on the subsequent stages of growth of the animals. Thus, the description of the behavior and physiological parameters of lactating piglets is of fundamental importance to propose management techniques best suited to the new genetic lines of pigs in commercial breeding (Ferreira *et al*., 2007b).

T_{bs} means in the first, second and third weeks of age for the piglets were 24.94±2.66 °C; 26.07±1.95 °C; and 25.29±1.89 °C, respectively (Figure 6A). Although the mean values of T_{bs} are contained within the environmental temperature range considered optimal for the second week of life of the piglets, which is 25 to 27 °C (Esmay, 1982; Nääs *et al*., 1998; Tolon and Nääs, 2005), it appears that, during 25.9% of the time (Figure 6B), the piglets were subject to temperatures outside the comfort range. The T_{bs} median for the first and third week of life ranged outside the ranges that are considered comfort, 27 to 32 °C and 22 to 24 °C, respectively (Esmay, 1982; Tolon and Nääs, 2005;. Nääs *et al*., 1998). However, the frequency of time within which T_{bs} stayed within the comfort range for the first and third week of life of the animals were 74.1 and 2.3%, respectively.

One of the advantages of the heating surface is to promote a more uniform temperature in the pig rest area than the heating by radiant energy (light bulbs), due to the floor-pig conduction process, as can be seen in the surveys conducted by Sabino *et al.* (2012) and Zhang

and Xin (2001). However, if the ambient temperature is too high, the piglets tend to spend less time in this environment. When this occurs, the piglets can be exposed to cold stress conditions, when they are affected by low temperatures in the external environment. This may bring risks to pigs, who may seek to warm up next to the pig's udder, being exposed to the area of crushing (Mores *et al*., 1998).

Pereira and Passos (1998), studying newborn piglets in which the variation in body temperature was monitored, concluded that the control of environmental temperature with the use of creep feeders and heating is indispensable to assist newborn piglets in maintaining their homeothermy, which was confirmed by Pandorfi (2002).

Regarding the RH, the median values of 77.40±6.99; 76.63±4.38; and 81.01±4.19% were observed for the first, second and third weeks, respectively (Figure 6C). When analyzing the RH frequencies of occurrence for the three weeks in question, it appears that, for more than 60% of the time (Figure 6D), the piglets were subjected to RH outside the comfort ranges for each week, and the ideal range should be 50% to 70% (Esmay, 1982; Nääs *et al*., 1998; Tolon and Nääs, 2005). According Nääs (1989), about 75% of body heat exchange with the environment takes place through conduction, convection and evaporation, it is important, therefore, that RH does not exceed 70%. Among the possible implications for animals, we can mention the commitment to homeothermy balance (Moura *et al*., 2010), which may cause dehydration of the

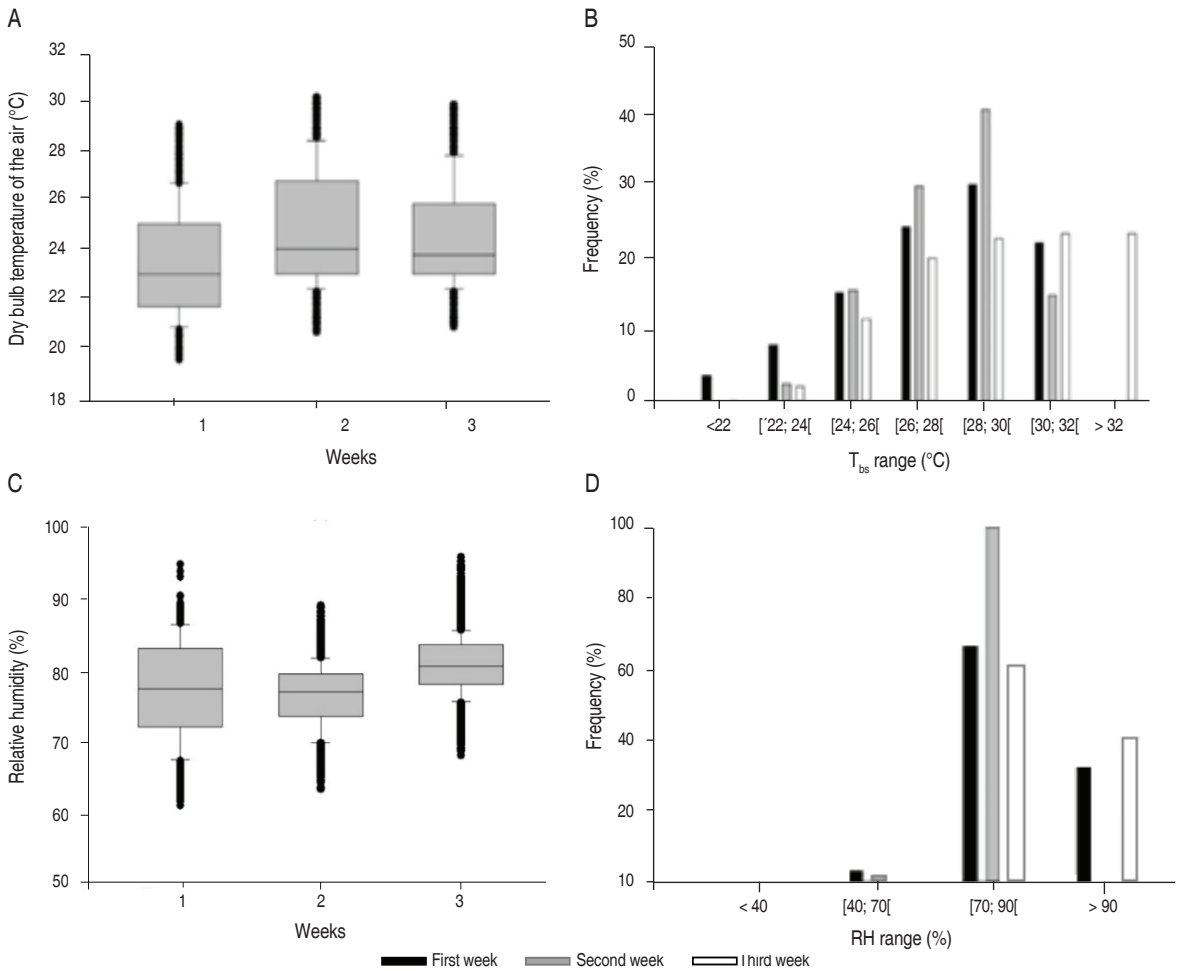

Figure 6. Box-plot and frequency of occurrence of A-B. The dry bulb temperature of the air and C-D. Relative humidity within the creep feeder in the first three weeks of life.

animals, reducing the productive performance, among other undesirable responses. Moreover, the high porcine metabolism associated with high ambient temperatures impedes heat dissipation.

Ferreira *et al.* (2007) reported that the normal rectal temperature of piglets in the first hours of life ranges around 37.8±0.3 °C. Given this, it can be inferred that piglets can have some physiological or pathological problem when present with rectal temperatures much above 37.8 °C. Taking the value of 37.8 °C as the upper limit for rectal temperature, from which the animal presents thermal problems, it can be seen that in a situation where the T_{bs} and RH are higher than 30 °C and 60%, respectively, piglets showed average values of RT (39.8 °C) much higher than those mentioned by the authors.

Predictions of WG with the fuzzy model showed that if the piglets were raised under ideal conditions of T_{bs} and RH during the 3 weeks, the weekly average WG would be 1.89, 3.52 and 4.38 kg, respectively, while in the farrowing house evaluated values of 2.01, 3.52 and 4.40 kg were observed.

CONCLUSIONS

The fuzzy model based on thermal design environment, characterized by the dry bulb air temperature (T_{bs}) and relative humidity (RH) developed was suitable for prediction of physiological responses and productive performance of piglets raised in farrowing houses with a solar heating system, with low standard deviation and high correlation with the measured data during the conduct of the field study. It can be used as a tool in making the decision to change the thermal environment, avoiding losses and providing better production rates. Because of the large amount of electricity that is used in this type of setting, there is a need for further research to minimize consumption without harming animal welfare while preserving the environment.

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REFERENCES

Aerts JM, Berckmans D and Schummans B. 1996. On-line measurement of bioresponses for climate control in animal production units. pp. 147-153. In: XI International Conference on Computers in Agriculture. American Society of Agricultural and Biological Engineering, Michigan, USA.

Amendola M, Castanho MJ, Nääs I and Souza AL. 2004. Análise matemática de condições de conforto térmico para avicultura usando a teoria dos conjuntos fuzzy. Biomatemática 14(1): 87-92.

Ayyub BM and Klir GJ. 2006. Uncertainty modeling and analysis in engineering and the sciences. Chapman & Hall/CRC, Boca Raton 378 p. doi: 10.1201/9781420011456

Carroll JA, Burdick NC, Coleman SW and Spiers DE. 2012. Influence of environmental temperature on the physiological, endocrine, and immune responses in livestock exposed to a provocative immune challenge. Domestic Animal Endocrinology 43: 146–153. doi: 10.1016/j.domaniend.2011.12.008

Carvalho LE, Pinheiro FML, Espíndola GB, Evangelista JNB and Vieira MMM. 2006. Ocorrência de diarreia em leitões submetidos a dietas com diferentes fontes de proteína de origem animal e vegetal no período de creche. V1 In: XVI Congresso Brasileiro de Zootecnia. Sociedade Brasileira de Zootecnia, Recife, Pernambuco, BR.

Carvalho CMC, Antunes RC, Carvalho AP and Caires RM. 2013. Bem-estar na suinocultura. Revista Eletrônica Nutritime 11(2): 2272-2286.

Castro JO, Campos AT, Ferreira RA, Yanagi JT and Tadeu HC. 2013. Uso de ardósia na construção de celas de maternidade para suínos: II - ambiente térmico e avaliação dos ruídos. Engenharia Agrícola 33: 37-45. doi: 10.1590/S0100-69162013000100005

CELESC. 2010. Aquecedor solar composto de produto descartáveis - Manual de construção e instalação. Em: http://www.celesc.com.br/portal/images/arquivos/manuais/manual-aquecedor-solar.pdf; consulta: novembro 2017.

Cornelissen AMG, Van Den Berg J, Koops WJ and Kaymak U. 2003. Elicitation of expert knowledge for fuzzy evaluation of agricultural production systems. Agriculture, Ecosystems & Environment 95(1): 1-18. doi: 10.1016/S0167-8809(02)00174-3

Esmay ML. 1982. Principles of animal environment. Second edition. AVI Publishing Company Inc, Westport, USA. 325 p.

Ferreira L, Yanagi JT, Nääs IA and Lopes MA. 2007a. Development of algorithm using fuzzy logic to predict estrus in dairy cows: Part 1. Agricultural Engeneering International: The CIGR Journal 9: 1-16.

Ferreira RA, Chiquieri J, Mendonça PP, Melo TV, Cordeiro MD and Soares RTR. 2007b. Comportamento e parâmetros fisiológicos de leitões nas primeiras 24 horas de vida. Ciência e Agrotecnologia 31(6): 1845-1849. doi: 10.1590/S1413-70542007000600036

Fernandes HC, Moreira RF, Longui FC, Rinaldi PC and Siqueira WC. 2011. Efeito do aquecimento e resfriamento de pisos no desempenho de matrizes e leitões. Revista Ceres 58(6): 701-709. doi: 10.1590/S0034-737X2011000600004

Firk R, Stamer E, Junge W and Krieter J. 2003. Improving oestrus detection by combination of activity measurements with information about previous oestrus cases. Livestock Production Science 82(1): 97-103. doi: 10.1016/S0301-6226(02)00306-8

Kim KY, Ko HJ, Kim HT, Kim CN and Byeon SH. 2008. Association between pig activity and environmental factors in pig confinement buildings. Australian Journal of Experimental Agriculture 48: 680–686. doi: 10.1071/EA06110

Gates RS, Chao K and Sigrimis N. 2001. Identifying design parameters for fuzzy control of staged ventilation control systems. Computers and Electronics in Agriculture 32(1): 61-74. doi: 10.1016/S0168-1699(00)00174-5

Goedseels V, Geers R, Tryen B, Wouters P, Goossens K, Villé H and Janssens S. 1992. A data acquisition system for electronic

identification, monitoring, and control of group-housed pigs. Journal of Agricultural Engineering 52(2): 25-33. doi: 10.1016/0021-8634(92)80048-W

Huynh TTT, Aarnink AJA, Verstegen MWA, Gerrits WJJ, Heetkamp MJW, Kemp B and Canh TT. 2005. Effects of increasing temperatures on physiological changes in pigs at different relative humidities. Journal Animal Science 83: 1385–1396. doi: 10.2527/2005.8361385x

Lacey B, Hamrita TK and Mcclendon R. 2000. Feasibility of using neural networks for a real-time prediction of poultry deep body temperature responses to stressful changes in ambient temperature. Applied Engineering in Agriculture 16(3): 303-308. doi: 10.13031/2013.5139

Le Dividich JL and Noblet J. 1981. Prise de colostum, thermoregulation et production de chalem chez le prcelet nouveané en relation avec le milieu thermique. Jounnée de lá Recherche Porcine en France 13: 11-16.

Leite MS, Fileti AMF and Silva FV. 2010. Desenvolvimento e aplicação experimental de controladores fuzzy e convencional em um bioprocesso. Revista Controle & Automação 21(2): 147-158. doi: 10.1590/S0103-17592010000200004

Lopes GT. 1999. Proposta de um controlador ótimo de altura da plataforma de corte e colhedoras. 1999. Tese (Doutorado em Engenharia Agrícola). Faculdade de Engenharia Agrícola. Universidade de Campinas, Campinas, São Paulo, BR. 155 p.

Martino JP. 1993. Technological forecasting for decision making. Third edition. McGraw-Hill Inc., New York. 461 p.

Medeiros BBL, Moura DJ, Massari JM, Carvalho TMR and Maia APA. 2014. Uso de geoestatística na avaliação de variáveis ambientais em galpão de suínos criados em sistema 'wean to finish' na fase de terminação. Engenharia Agrícola 34(5): 800-811. doi: 10.1590/S0100-69162014000500001

Montanholi YR, Odongo NE, Swanson KC, Schenkel FS, Mcbride BW and Miller SP. 2008. Application of infrared thermography as an indicator heat and methane production and its use in the study of skin temperature in response to physiological events in dairy cattle (*Bos taurus*). Journal of Thermal Biology 33(8): 468-475. doi: 10.1016/j.jtherbio.2008.09.001

Mores N, Sobestiansky J, Wentz I and Moreno AM. 1998. Manejo do leitão desde o nascimento até o abate. Cap. 7. pp. 135-161. In: Mores N; Sobestiansky J; Wentz Ivo; Moreno AM (eds.). Suinocultura intensiva: produção, manejo e saúde do rebanho. Serviço de Produção de Informação – SPI. Embrapa, Brasília. Cap. 7.

Moura DJ, Bueno LGF, Lima KAO, Carvalho TMR and Maia APAM. 2010. Strategies and facilities in order to improve animal welfare. Revista Brasileira de Zootecnia 39: 311-316. doi: 10.1590/S1516-35982010001300034

Nääs IA. 1989. Princípios de conforto térmico na produção animal. Editora Ícone, São Paulo. 183 p.

Nääs IA. 1998. Biometeorologia e construções rurais em ambiente tropical. pp. 63-73. In: II Congresso Brasileiro de Biometeorologia. Sociedade Brasileira de Biometeorologia, Goiânia, Goiás, Br.

Nascimento G, Pereira Df, Nääs IA and Rodrigues LHA. 2011. Índice fuzzy de conforto térmico para frangos de corte. Engenharia Agrícola 31(2): 219-229. doi: 10.1590/S0100-69162011000200002

Pandorfi H. 2002. Avaliação do comportamento de leitões em diferentes sistemas de aquecimento por meio da análise de imagem e identificação eletrônica. Dissertação (Mestrado em Física do Ambiente Agrícola). Escola Superior de Agricultura "Luiz de Queiroz". Universidade de São Paulo, Piracicaba, São Paulo, Br. 89 p.

Pandorfi H. 2005. Comportamento bioclimático de matrizes suínas em gestação e o uso de sistemas inteligentes na caracterização do ambiente produtivo: suinocultura de precisão. Tese (Doutorado em Física do Ambiente Agrícola). Escola Superior de Agricultura "Luiz de Queiroz". Universidade de São Paulo, Piracicaba, São Paulo, Br. 119 p.

Pandorfi H, Silva IJO, Guiselini C and Piedade SMS. 2007. Uso da lógica fuzzy na caracterização do ambiente produtivo para matrizes gestantes. Engenharia Agrícola 27(1): 83-92. doi: 10.1590/S0100-69162007000100001

Perdomo CC, Sobestiansky J, Oliveira PVA and Oliveira JA. 1987. Efeito de diferentes sistemas de aquecimento no desempenho de leitões. EMBRAPA–CNPSA, Concórdia, Embrapa, Br. 122: 1-3.

Pereira C and Passos A. 1998. Informe técnico: cuidados especiais. Revista Suinocultura Industrial [S.l.], 134: set.

Preble JF. 1984. The selection of Delphi panels for strategic planning purposes. Strategic Management Journal 5(2): 157-170. doi: 10.1002/smj.4250050206

Queirós MPG and Nääs IA. 2005. Estimativa de padrão de conforto ambiental para creche de suínos usando lógica fuzzy. In: V Congresso Brasileiro de Agroinformática, Sociedade Brasileira de Agroinformática, Londrina, Paraná, Br.

Ribacionka F. 1999. Sistemas computacionais baseados em lógicas fuzzy, 1999. Dissertação (Mestrado em Engenharia Elétrica). Universidade Mackenzie, São Paulo, Br. 115 p.

Sabino LA, Abreu PG, Sousa Júnior VR, Abreu VM and Lopes LS. 2012. Comparação de dois modelos de escamoteadores sobre o desempenho dos leitões. Acta Scientiarum, Animal Sciences 34(1): 21-25. doi: 10.4025/actascianimsci.v34i1.11675

Schiassi L, Yanagi JT, Damasceno FA, Saraz JAO and Machado NS. 2012. Fuzzy modeling applied to the welfare of poultry farms workers. Dyna 79: 127-135.

Tavares GF and Schiassi L. 2016. Modelagem fuzzy como ferramenta para predição do ganho de peso diário para frangos de corte. Journal Animal Behavior Biometeorol 4(2): 32-38. doi: 10.14269/2318-1265/jabb.v4n2p32-38

Tolon YB and Nääs IA. 2005. Avaliação de tipos de ventilação em maternidade de suínos. Engenharia Agrícola 25(3): 565-574.

Turco SHN, Ferreira AS, Oliveira RFM, Aguiar MA, Cecon PR and Araújo GGL. 1998. Desempenho de porcas e leitões em maternidades com diferentes sistemas de acondicionamento térmico no inverno. Revista Brasileira de Zootecnia 27(5): 988-993.

Weber L and Klein PAT. 2003. Aplicações de lógica fuzzy em sofware e hardware. Editora da ULBRA, Canoas. 112 p.

Xie Q, Ni J and Su Z. 2017. A prediction model of ammonia emission from a fattening pig room based on the indoor concentration using adaptive neuro fuzzy inference system. Journal of Hazardous Materials 325: 301-309. doi: 10.1016/j.jhazmat.2016.12.010

Xie Q, Ni J and Su Z. 2017. Fuzzy comprehensive evaluation of multiple environmental factors for swine building assessment and control. Journal of Hazardous Materials 340: 463-471. doi: 10.1016/j.jhazmat.2017.07.024

Yanagi JT, Xin H, Gates RS and Ferreira L. 2006. Fuzzy logic model to predict laying hen body temperature rise during acute heat stress. pp. 1-4. In: XXXV Congresso Brasileiro de Engenharia Agrícola. Associação Brasileira de Engenharia Agrícola, João Pessoa, Paraíba, Br.

Yanagi JT, Schiassi L, Abreu LHP, Barbosa JÁ and Campos AT. 2012. Procedimentos fuzzy aplicado à avaliação da insalubridade em atividades agrícolas. Engenharia Agrícola 32(3): 423-434. doi: 10.1590/S0100-69162012000300002

Zhang Q and Xin H. 2001. Responses of piglets to creep heat type and location in farrowing crate. Applied Engineering in Agriculture 17(4): 515-519. doi: 10.13031/2013.6467

Research article

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Behavior of bioactive compounds and antioxidant activity of mango (Azucar cultivar) juice during storage at 4 °C

Comportamiento de compuestos bioactivos y actividad antioxidante del jugo de mango (variedad Azúcar) durante el almacenamiento a 4 °C

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ABSTRACT

Mango (*Mangifera indica* L.) is one the fruits that have shown antioxidant activity and high nutritional value. It was evaluated the effect of storage time and temperature on polyphenol content, ascorbic acid and antioxidant activity of mango (cv. Azucar) juice stored up to 64 days at 4 °C. Total polyphenol content was measured by Folin-Ciocalteu method, mangiferin and ascorbic acid were measured by HPLC (High-Performance Liquid Chromatography) and antioxidant activity was measured by ORAC (Oxygen Radical Absorbance Capacity), and ABTS•+ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) method. Total phenolic content decreased after 16 days of juice storage. Ascorbic acid values did not show significant differences until 48 days of storage, and mangiferin content was very similar throughout storage time. The antioxidant activity measured by ORAC method was similar until the end of the storage; however, ABTS value decreased after 32 days of juice storage. In conclusion, storage up to 32 days of mango juice at 4 °C did not alter its antioxidant activity and ascorbic acid content.

Keywords:
Antioxidant
Ascorbic acid
cv. Azucar
Mangiferin
Mango juice
Polyphenols

RESUMEN

El mango (*Mangifera indica* L.) es una de la frutas que ha mostrado actividad antioxidante y un valor nutricional alto. Se evaluó el efecto del tiempo de almacenamiento y la temperatura, en el contenido fenólico total, niveles de ácido ascórbico y actividad antioxidante, de un jugo de mango (variedad Azúcar) almacenado a 4 °C durante 64 días. El contenido fenólico total fue medido por el método de Folin-Ciocalteau, los niveles de mangiferina y ácido ascórbico fueron medidos mediante HPLC (cromatografía líquida de alta eficacia) y la actividad antioxidante fue medida mediante ORAC (capacidad de absorbancia del radical oxígeno) y ABTS•+ (ácido 2,2'-azinobis- (3-etilbenzotiazolin-6-sulfónico). El contenido fenólico total disminuyó después de 16 días de almacenamiento del jugo. Los niveles de ácido ascórbico no mostraron cambios significativos hasta el día 48 de almacenamiento, y el contenido de mangiferina mostró valores similares durante el tiempo de almacenamiento. La actividad antioxidante medida por el método de ORAC no mostró variaciones significativas durante el tiempo de almacenamiento, en contraste se observó una disminución de los valores de ABTS después del día 32 de almacenamiento. En conclusión, el almacenamiento del jugo de mango (variedad Azúcar) durante 32 días a 4 °C no da lugar a cambios en su actividad antioxidante o contenido de ácido ascórbico.

Palabras clave:
Antioxidante
Ácido ascórbico
var. Azúcar
Mangiferina
Jugo de mango
Polifenoles

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High consumption of fruit and vegetables has been associated with protection against chronic diseases such as cancer, cardiovascular and neurodegenerative diseases (Slavin and Lloyd, 2012; Zhang *et al*., 2014). These beneficial effects of fruits and vegetables have been attributed partly to the antioxidant activity of their phytochemical constituents (Wojdyło *et al*., 2014).

Mango (*Mangifera indica* L.) is a popular tropical fruit due to its taste, aroma, and flavor; it is cultivated in more than 100 tropical and subtropical countries (Yang *et al*., 2013). There are thousands of mangoes cultivars worldwide (Liu *et al*., 2013) and the most important mango Colombian cultivars in terms of production are the cultivars Hilacha, Tommy, Keitt, and Azucar; being the latter currently import into the United States (US) and Europe on a large scale (Krenek *et al*., 2014).

Mango has high nutritional value and is an important source of dietary antioxidants such as carotenoids, ascorbic acid and phenolic compounds (Manthey and Perkins, 2009). The phenolic compounds include flavonoids, phenolic acids, xanthones and gallotannins (Kim *et al*., 2010), although this composition changes between different mango cultivars (Kim *et al*., 2010; Manthey and Perkins, 2009).

Among the compounds mentioned above, special interest has been focused on ascorbic acid and mangiferin. The ascorbic acid also known as vitamin C is an important micronutrient in the human diet involved in the antioxidant defense, protecting proteins and lipid membranes from oxidative damage caused by Reactive Oxygen Species (ROS) due to its reducing capacity, and it is also known as neuroprotective agent (Du *et al*., 2012).

Mangiferin is a C-glucosyl xanthone which can be found in several plants such as *Mangifera indica* (Negi *et al*., 2013); it is predominantly found in peel and stem bark of mango fruit. Mangiferin contribute to prevent fenton reaction and lipid peroxidation because its catechol motive can forms a stable complex with Fe^{3+} (Benard and Chi, 2015). Mangiferin also has a potent capacity to neutralize ROS, such as peroxyl radical, hydroxyl radical, hydrogen peroxide, superoxide anion, and its activity is similar to ascorbic acid (Benard and Chi, 2015).

Considering that mango is a seasonal fruit with a short shelf life, its processing is important for consumers to ensure a longer life (Alikhani, 2014). Therefore, mango fruit is processed into several products such as puree, nectar, and juice (Appiah *et al*., 2011); these products should be kept by consumers between 0 to 4 °C to prevent their spoilage (Kaddumukasa *et al*., 2017); however, there are few studies about changes in its bioactive compounds and antioxidant activity during storage time at that temperature.

Currently, consumers prefer foods which may have a positive impact on their health. Therefore, it is important to consider the effect of juice storage time on bioactive compounds and antioxidant activity because this may affect consumers acceptance (Beh *et al*., 2012). Thus, it was evaluated the changes of ascorbic acid, total phenols and antioxidant activity of mango (cv. Azucar) juice stored up to 64 days at 4 °C; besides, we investigated if mango juice had mangiferin, considering it has been proposed as one of the most important antioxidants of mango (Matkowski *et al*., 2013).

MATERIALS AND METHODS

Raw material

Mature mango (cv. Azucar) grown in Colombia's Caribbean coast was purchased from a local market in Medellin, Colombia (June 2015). Mango got ripened after storage at 25 °C, and ripeness degree was determined according to the following criteria: peel color, firmness, and flavor based on Colombian Technical Norm (NTC *by its initials in Spanish*) 5139 (Zapata *et al*., 2017). Ripe mango was immersed in a solution of sodium hypochlorite (100 ppm), washed with water, peeled and cut into small pieces which were used immediately to prepare the juice.

Juice preparation

The juice was prepared as described in a previous study with some modifications (Zapata *et al*., 2017). The juice was prepared by mixing mango pieces and water (1:4) in a blender for several minutes; the total soluble solids of the juice were measured at 20 °C using a refractometer. Xanthan gum (0.07%) was added to the juice as stabilizer. Finally, juice was pasteurized at 85 °C for 10 min, sweetened with 2 g L^{-1} of sucralose, and packaged into low-density polyethylene bag and immediately stored at 4 °C until use.

Determination of total phenolic content

The total phenolic content was determined according to a modified Folin–Ciocalteu described by Prior *et al*. (2005). The juice (10 µL) was mixed with 125 µL of Folin–Ciocalteu reagent and 400 µL of sodium carbonate solution (7.1% w/v); the resulting solution was brought up to a final volume of 1000 µL. The mixture was incubated at room temperature for 30 min in the dark. The absorbance was measured at 760 nm against a blank. Standard solution of gallic acid was used to perform calibration curves. The results were expressed as mg of Gallic Acid Equivalents, mg GAE L^{-1}.

Determination of ascorbic acid

Ascorbic acid was quantified using a Shimadzu Prominence (LC-20AD) HPLC system equipped with autosampler (SIL-20A/HT) and PDA detector (SPD 6AUV) in juice stored up to 64 days at 4 °C. Juice was filtered through cellulose membrane filter (0.45 µm), and then gradient separations were done using Lichrospher (Merck) RP C18 column (5 µm, 250x4 mm) at 35 °C; gradually, 20 µL of juice was injected using autosampler. The mobile phase was 0.1% formic acid in water run at 0.8 mL min^{-1} under isocratic conditions. Ascorbic acid was quantified at 245 nm using a calibration curve, and the results were expressed as mg ascorbic acid L^{-1} (Shakya and Navarre, 2006).

Determination of mangiferin

Shimadzu Prominence (LC-20AD) HPLC system described previously was used for determination of mangiferin in juice stored up to 64 days at 4 °C. The flow rate was 0.6 mL min^{-1} and the injection volume was 10 µL. Analysis was carried out with Lichrospher RP C18 column (5 µm, 250x4 mm) using a gradient elution of 0-1 min 5% solvent B (5% (v/v) acetic acid in water with acetonitrile 50:50), 2-10 min 5-25% solvent B, 10-40 min 25-55% solvent B, 40-45 min 55-90% solvent B, 45-50 min 90-55% solvent B, 50-55 min 55-5% solvent B, 55-60 min 5% solvent B, and solvent A was 2% (v/v) acetic acid in water. Column temperature was 30 °C and mangiferin was determined at 258 nm (Luo *et al*., 2012). The determination was done only one time.

Antioxidant activity determined by ORAC (Oxygen Radical Absorbance Capacity) assay

ORAC assay was performed as described by Ou *et al*. (2001) with some modifications, in mango juice stored up to 64 days at 4 °C. The working solution was prepared by mixing 21 µL of 10 µM fluorescein solution, 2899 µL of 75 mM phosphate buffer (pH 7.4), 50 µL of 600 mM AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride) and 30 µL of juice. Fluorescence was measured on a Perkin Elmer LS45 spectrofluorometer with a thermostatted multicell. The results were expressed as µM Trolox® L^{-1}, according to the following equation:

$$ORAC = \frac{(AUC - AUC^{\circ})}{(AUC_{Trolox} - AUC^{\circ})} f[Trolox]$$

Where:

AUC: is the area under the curve of the sample.

AUC°: is the area under the curve for the control

AUC_{Trolox}: is the area under the curve for Trolox

f : is the dilution factor for juice = 24.

Antioxidant activity determined by ABTS•+ assay

10 µL of juice was added gradually to 990 µL of diluted ABTS•+ (2,2´-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) in phosphate buffer (pH 7.4), and the resulting solution was incubated at room temperature for 30 min in the dark. The absorbance was measured at 734 nm against a blank. Trolox standard solution was used to perform the calibration curves, and the results were expressed as µM Trolox L^{-1} (Re *et al*., 1999).

Kinetic model

It was performed an adjustment model to determine the kinetic behavior of antioxidant activity and mango components during storage and to estimate the rate of decrease of mango components during the storage.

Statistics

Assays were conducted by triplicate and data were reported as the mean±standard deviation (SD). The differences between groups were estimated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A *P*-value less than 0.05 ($P<0.05$) was considered statistically significant. The results were analyzed using GraphPad Prism software version 6.0. A regression model was found to estimate the kinetic behavior of antioxidant activity and mango components throughout time.

RESULTS AND DISCUSSION

Juice

The juice fruit content was 18%, and its total soluble solids were 8 °Brix.

Total phenolic content

The values of total phenols showed a reduction during juice storage time (Figure 1). The highest values of total phenolic content were observed up to 16 days of juice storage corresponding to 126.5±7.9 and 123.9±4.5 mg GAE L^{-1}, respectively and they did not show significant differences between them. After 32 days of storage, juice showed a decrease in its phenolic content, and it was significantly different in comparison to values observed at the beginning of the storage. The lowest total phenols value was observed at the end of the juice storage, corresponding to 96.23±3.25 mg GAE L^{-1}.

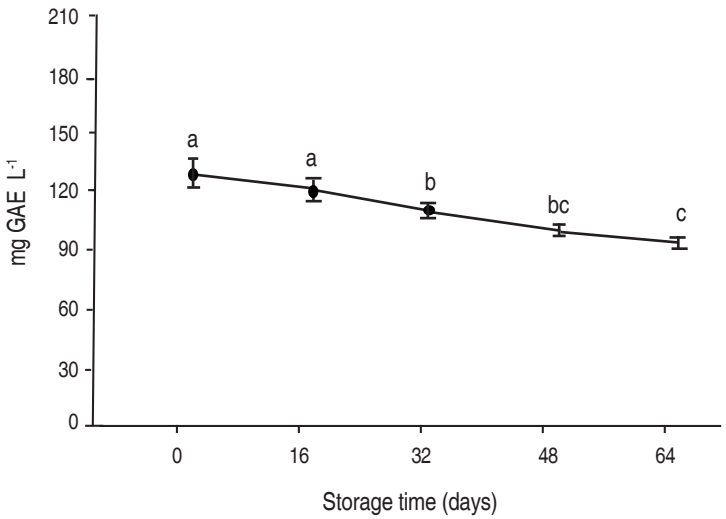

Figure 1. Effect of storage time and temperature on the total phenolic content of mango juice. Values are the means ± standard deviation of three replicates. The same small letters indicate not significant statistically difference (*P*>0.05). Tukey's post-hoc test. GAE: Gallic Acid Equivalents.

These results agree with previous studies which have observed a decrease phenolic compounds in juices after 20 days at 4 °C; this could be explained by polyphenol oxidase activity which reduces phenolic compounds (Mizobutsi *et al*., 2010).

Total phenolic content values up to 48 days found in the present study were higher than those reported by Beh *et al*. (2012) which found a total phenolic content of 9.26 mg GAE L^{-1} in fresh juice prepared with mango from Malaysia. While, total phenolic content value was similar to the value reported by Abdullakasim *et al*. (2007), corresponding to 100 mg GAE L^{-1} in fresh juice prepared with mango from Thailand. However, some authors have reported higher phenolic content in juice prepared with mango from Iran, Malaysia and Algeria corresponding to 567.2, 804.00 and 413.5 mg GAE L^{-1}, respectively (Mahdavi *et al*., 2010; Saci *et al*., 2015; Wern *et al*., 2016).

Ascorbic acid content

Another important component of mango is the ascorbic acid which is known as a potent antioxidant. Therefore, their levels in juice were determined. Ascorbic acid in the juice was quite stable during most of the evaluated days, and its content was within a range of 9.30 to 16.35 mg ascorbic acid L^{-1} (Figure 2). Ascorbic acid values did not show significant differences between different storage time compared to day 1, except for juice stored for 64 days, which showed the lowest ascorbic acid value corresponding to 9.3±0.14 mg ascorbic acid L^{-1}. Ascorbic acid decrease at the end of the storage could be attributed to an ascorbic acid degradation induced by light, oxygen and ascorbate oxidase (Castro *et al*., 2016; di Venere *et al*., 2011). Moreover, ascorbic acid values showed by mango (cv. Azucar) juice were lower than those reported by Mahdavi *et al*. (2010) for a fresh juice formulated with Mango from Tabriz-Iran, corresponding to 146.5 mg L^{-1}.

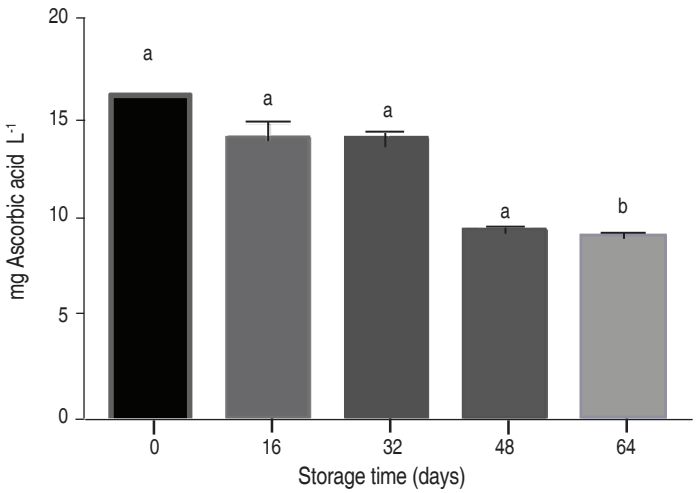

Figure 2. Ascorbic content of mango juice stored up to 64 days at 4 °C.

The variation of ascorbic acid and phenolic content depends on several factors such as cultivar, harvest date, ripeness, processing techniques and location of mango fruit (Manthey and Perkins, 2009).

Mangiferin content

Mangiferin content in mango (cv.Azucar) juice was evaluated by HPLC-DAD. At the beginning of the storage, the juice showed the highest mangiferin levels corresponding to 11.50 mg L^{-1} (Figure 3A) detected at 258 nm with a retention time of 4.3 min. After 64 days of storage, the juice showed the lowest mangiferin levels (8.33 mg L^{-1}). Mangiferin levels were 9.21, 8.64 and 8.54 mg L^{-1} after 16, 32 and 48 days of storage, respectively (data not shown). Mangiferin standard showed a retention time of 4.3 min (Figure 3B).

Mangiferin content was similar over the storage time and this is an important finding considering it has shown a strong antioxidant activity in vitro (Matkowski *et al*., 2013). Mangiferin is also able to reduce induced oxidative stress in rats' brain (Márquez *et al*., 2012).

Antioxidant activity

It was determined the antioxidant activity in a mango juice stored up to 64 days by two different methods ORAC and ABTS•+. ORAC values were similar from the beginning to the end of juice storage, and they did not show significant differences between them. The highest and the lowest ORAC values were observed at the beginning and the end of the storage, corresponding to 2563.16±129.36 and 1933.16±104.35 μmol Trolox L^{-1}, respectively (Table 1).

The scavenging activity of mango juice against ABTS radical was similar until 32 days of storage compared to the juice stored for 1 day at 4 °C, and it did not show significant differences between them (Table 1). After 16 days of juice storage, it was observed the highest ABTS•+ value, corresponding to 656.57±12.35 μmol Trolox L^{-1} and the lowest value was shown after 64 days of storage, corresponding to 533.60±14.69 μmol Trolox L^{-1}. Antioxidant activity measured by ABTS•+ assay decreased after 32 days of juice storage and showed significant differences compared to ABTS•+ values shown by juice stored during 1 day at 4 °C.

Antioxidant activity has been associated with phenolic content (Wojdyło *et al*., 2014). However, other components of mango such as ascorbic acid and carotenoids are also known as potent antioxidants (Fiedor and Burda, 2014; Manthey and Perkins, 2009). Therefore, the antioxidant activity observed in this study cannot be only attributed to its phenolic compounds, but also the activity of the several antioxidants present in the juice and the synergistic activity between them.

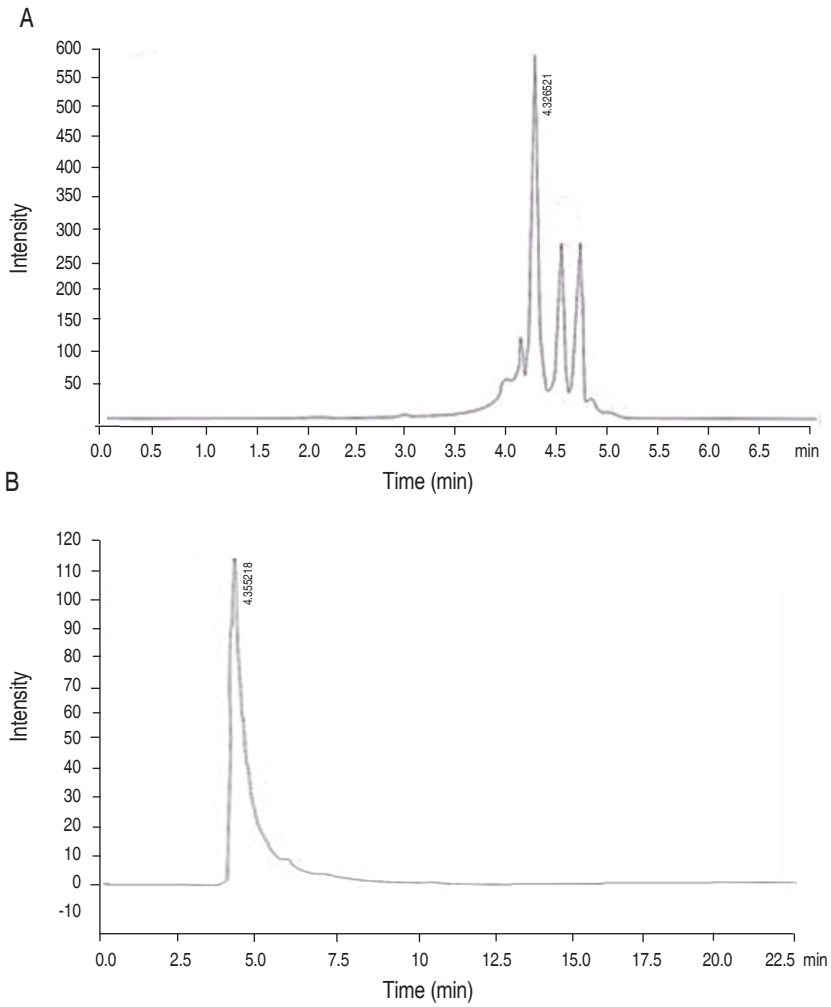

Figure 3. Identification of mangiferin by HPLC. A. Mango (cv. Azúcar) juice; B. Mangiferin standard.

Table 1. Antioxidant activity of the mango juice. The same small letters indicate not significant statistically difference (P>0.05). Tukey's post-hoc test.

Juice storage time (days)	ABTS (µmol Trolox L^{-1})	ORAC (µmol Trolox L^{-1})
1	632.71±19.05[a]	2563.16±129.36[a]
16	656.57±12.35[a]	2551.96±120.18[a]
32	632.37±18.42[a]	2335.43±124.76[a]
48	591.96±6.59[b]	2275.18±128.03[a]
64	533.60±14.69[b]	1933.16±104.35[a]

Kinetic model

Table 2 presents the kinetic behavior of mangiferin, polyphenols, ascorbic acid and antioxidant activity of mango juice stored at 4 °C. There was a relationship between time and mangiferin, ascorbic acid and antioxidant activity values. Mangiferin showed more susceptibility to

degradation over time with respect to total phenols and ascorbic acid in the juice. Regarding to antioxidant activity, ABTS values decreased faster than ORAC values indicating that molecules with capacity to neutralize free radicals through hydrogen atom transfer (ORAC mechanism) were more stable during storage at 4 °C, compared to molecules which reduces free radicals through electron transfer (ABTS mechanism) (Schaich *et al.*, 2015).

Table 2. Kinetic modeling parameters of mango juice stored at 4 °C.

Regression variables	Regression equation	Model type	R^2
Mangiferin vs. time	$mg\ mangiferin\ L^{-1} = 11.454 - 0.733\,Ln(days)$	Logarithmic	0.9931
Polyphenols vs. time	$mg\ GAE\ L^{-1} = 128.448 - 0.518 * days$	Linear	0.9657
ABTS vs. time	$\mu mol\ Trolox\ L^{-1} = 651.938 - 0.0273 * days^2$	Quadratic	0.9334
Ascorbic acid vs. time	$mg\ Asc.acid L^{-1} = 16.5318 - 0.1184 * days$	Linear	0.9008
ORAC vs. time	$\mu mol\ Trolox\ L^{-1} = 2645.9 - 9.755 * days$	Linear	0.8996

CONCLUSIONS

This study found that storage time and temperature influence on composition and antioxidant activity of mango (cv. Azucar) juice. Although total phenolic content decreased after 16 days at 4 °C, antioxidant activity measured by two methods remained stable until 32 days of storage; also, ascorbic acid and mangiferin content were very similar until near the end of the storage time. Considering that mango juice has antioxidant activity and it is a good source of antioxidants, it could potentially help to prevent oxidative stress in vivo.

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REFERENCES

Abdullakasim P, Songchitsomboon S, Techagumpuch M, Balee N, Swatsitang P and Sungpuag P. 2007. Antioxidant capacity, total phenolics and sugar content of selected Thai health beverages. International Journal of Food Sciences and Nutrition 58(1): 77–85. doi: 10.1080/09637480601140946

Alikhani M. 2014. Enhancing safety and shelf life of fresh-cut mango by application of edible coatings and microencapsulation technique. Food Science and Nutrition 2(3): 210–217. doi: 10.1002/fsn3.98

Appiah F, Kumah P and Idun I. 2011. Effect of ripening stage on composition, sensory qualities and acceptability of keitt mango (*Mangifera indica* L.) chips. African Journal of Food, Agriculture, Nutrition and Development 11(5): 5096–5109. doi: 10.4314/ajfand.v11i5.70439

Beh LK., Zakaria Z, Beh BK, Ho WY, Yeap SK., Banu N and Alitheen NBM. 2012. Comparison of total phenolic content and antioxidant activities of freeze-dried commercial and fresh fruit juices. Journal of Medicinal Plants Research 6(48), 5857–5862. doi: 10.5897/JMPR11.991

Benard O and Chi Y. 2015. Medicinal Properties of Mangiferin, Structural Features, Derivative Synthesis, Pharmacokinetics and Biological Activities. Mini-Reviews in Medicinal Chemistry 15(7): 582–594. doi: 10.2174/1389557515666150401111410

Castro C, Sánchez EJ, Saucedo S, Rojas R, Aranda J and Martínez GCG. 2016. Fluctuations in phenolic content, ascorbic acid and total carotenoids and antioxidant activity of fruit beverages during storage. Heliyon 2(9): e00152. doi: 10.1016/j.heliyon.2016.e00152

di Venere A, Nicolai E, Rosato N, Rossi A, Finazzi Agrò A and Mei G. 2011. Characterization of monomeric substrates of ascorbate oxidase. FEBS Journal 278(9): 1585-1593. doi: 10.1111/j.1742-4658.2011.08084.x

Du J, Cullen JJ and Buettner GR. 2012. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochimica et Biophysica Acta - Reviews on Cancer 1826(2): 443-457. doi: 10.1016/j.bbcan.2012.06.003

Fiedor J and Burda K. 2014. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 6(2): 466–488. doi: 10.3390/nu6020466

Kaddumukasa PP, Imathiu SM, Mathara JM and Nakavuma JL. 2017. Influence of physicochemical parameters on storage stability: Microbiological quality of fresh unpasteurized fruit juices. Food Science and Nutrition 5(6): 1098–1105. doi: 10.1002/fsn3.500

Kim H, Moon JY, Kim H, Lee DS, Cho M, Choi HK, Kim YS, Mosaddik A and Cho SK. 2010. Antioxidant and antiproliferative activities of mango (*Mangifera indica* L.) flesh and peel. Food Chemistry 121(2): 429–436. doi: 10.1016/j.foodchem.2009.12.060

Krenek KA, Barnes RC and Talcott ST. 2014. Phytochemical composition and effects of commercial enzymes on the hydrolysis of gallic acid glycosides in mango (*Mangifera indica* L. cv. 'Keitt') pulp. Journal of Agricultural and Food Chemistry 62(39): 9515–9521. doi: 10.1021/jf5031554

Liu FX, Fu SF, Bi XF, Chen F, Liao XJ, Hu XS and Wu JH. 2013. Physico-chemical and antioxidant properties of four mango (*Mangifera indica* L.) cultivars in China. Food Chemistry 138(1): 396–405. doi: 10.1016/j.foodchem.2012.09.111

Luo F, Lv Q, Zhao Y, Hu G, Huang G, Zhang J, Sun C, Li Xian and Chen K. 2012. Quantification and purification of mangiferin from Chinese mango (*Mangifera indica* L.) cultivars and its protective effect on human umbilical vein endothelial cells under H_2O_2-induced stress. International Journal of Molecular Sciences 13(9): 11260–11274. doi: 10.3390/ijms130911260

Mahdavi R, Nikniaz Z, Rafraf M and Jouyban A. 2010. Determination and comparison of total polyphenol and vitamin C contents of natural fresh and commercial fruit juices. Pakistan Journal of Nutrition 9(10): 968–972. doi: 10.3923/pjn.2010.968.972

Manthey JA and Perkins P. 2009. Influences of harvest date and location on the levels of β-carotene, ascorbic acid, total phenols, the in vitro antioxidant capacity, and phenolic profiles of five commercial varieties of mango (*Mangifera indica* L.). Journal of Agricultural and Food Chemistry 57(22): 10825–10830. doi: 10.1021/jf902606h

Márquez L, García B, Madrigal JLM and Leza JC. 2012. Mangiferin decreases inflammation and oxidative damage in rat brain after stress. European Journal of Nutrition 51(6): 729–739. doi: 10.1007/s00394-011-0252-x

Matkowski A, Kus P, Goralska E and Wozniak D. 2013. Mangiferin - a bioactive xanthonoid, not only from mango and not just antioxidant. Mini-Reviews in Medicinal Chemistry 13(3): 439–455. doi: 10.2174/1389557511313030011

Mizobutsi GP, Finger FL, Ribeiro RA, Puschmann R, De Melo LL and Ferreira W. 2010. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. Scientia Agricola 67(2): 213–217. doi: 10.1590/S0103-90162010000200013

Negi JS, Bisht VK, Singh P, Rawat MSM and Joshi GP. 2013. Naturally Occurring Xanthones: Chemistry and Biology. Journal of Applied Chemistry 2013(1): 1–9. doi: 10.1155/2013/621459

Ou B, Hampsch M and Prior RL. 2001. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of Agricultural and Food Chemistry 49(10): 4619–4626. doi: org/10.1021/jf010586o

Prior RL, Wu X and Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53(10): 4290-302. doi: 10.1021/jf0502698

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26(9–10): 1231–1237. doi: 10.1016/S0891-5849(98)00315-3

Saci F, Meziant L and Louaileche H. 2015. Effect of Storage Time and Temperature on the Health-Promoting Substances and Antioxidant Activity of Two Commercial Fruit Based-Beverages. International Journal of Bioinformatics and Biomedical Engineering 1(2): 118–122.

Shakya R and Navarre DA. 2006. Rapid screening of ascorbic acid, glycoalkaloids, and phenolics in potato using high-performance liquid chromatography. Journal of Agricultural and Food Chemistry 54(15): 5253–5260. doi: 10.1021/jf0605300

Slavin JL and Lloyd B. 2012. Health Benefits of Fruits and Vegetables. Advances in Nutrition 3(4): 506–516. doi: 10.3945/an.112.002154

Schaich KM, Tian X and Xie J. 2015. Hurdles and pitfalls in measuring antioxidant efficacy: A critical evaluation of ABTS, DPPH, and ORAC assays. Journal of Functional Foods 18: 782–796. doi: 10.1016/j.jff.2015.05.024

Wern KH, Haron H and Keng CB. 2016. Comparison of Total Phenolic Contents (TPC) and antioxidant activities of fresh fruit juices, commercial 100% fruit juices and fruit drinks. Sains Malaysiana 45(9): 1319–1327.

Wojdyło A, Teleszko M and Oszmiański J. 2014. Antioxidant property and storage stability of quince juice phenolic compounds. Food Chemistry 152: 261–270. doi: 10.1016/j.foodchem.2013.11.124

Yang M, Chung S, Floegel A, Song W, Koo S and Chun O. 2013. Dietary antioxidant capacity is associated with improved serum antioxidant status and decreased serum C-reactive protein and plasma homocysteine concentrations. European Journal of Nutrition 52(8): 1901–11. doi: 10.1007/s00394-012-0491-5

Zapata MB, Chaparro D, Rojano BA, Alzate AF, Restrepo LF and Maldonado ME. 2017. Effect of storage time on physicochemical, sensorial, and antioxidant characteristics and composition of mango (cv. Azucar) juice. Emirates Journal of Food and Agriculture 29(5): 367–377. doi: 10.9755/ejfa.2016-09-1256

Zhang PY, Xu X and Li XC. 2014. Cardiovascular diseases: oxidative damage and antioxidant protection. European Review for Medical and Pharmacological Sciences 18(20): 3091–3096.

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Survival, growth and photosynthesis analysis of native forest species established in the tropical dry forest in Antioquia, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Análisis de supervivencia, crecimiento y fotosíntesis de especies forestales nativas en el bosque seco tropical en Antioquia, Colombia

doi: 10.15446/rfnam.v72n1.69575

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ABSTRACT

The tropical dry forest (TDF) is one of the most affected ecosystems by anthropic activities in the world; so, it is necessary to study the dynamics of its ecosystem in order to restore it. With the aim of determining the survival, development, and photosynthetic behaviors of forest species at a young age, a field study was performed using three species *Cedrela odorata* L. (Spanish cedar), *Pachira quinata* (Jacq.) W.S. Alverson (red ceiba) and *Ochroma pyramidale* (Cav. ex Lam.) Urb. (balsa) species. Field data were collected in different periods whose climatic conditions were: dry period (S.0), first rainy period (Ll.1), first dry period (S.1), second rainy period (Ll. 2), and second dry period (S.2). The total height (H) and the root collar diameter (RCD) were measured repeatedly, and two harvests were made to measure dry weight. Besides, photosynthetic performance and its effect on the species development species during three contrasting rainfall periods was evaluated by measuring photosynthetically active radiation (PAR), stomatal conductance (g_s), intercellular carbon (C_{int}), net photosynthesis (NP), transpiration (t_{rans}), efficient water use (EWU) and efficient light use (ELU) from 8:00 and 17:00 h during the day. Analysis of variance was performed obtaining significant differences (P<0.05) in the interaction time×species regarding variables H and RCD, and the photosynthetic variable NP. The g_s and t_{rans} variables showed statistical significance with the species and rainfall periods; C_{int} was significant only for the rainfall periods. The species *O. pyramidale* presented the best survival and tolerance to weather by adapting physiological mechanisms, while *C. odorata* was the most affected species by climatic conditions concerning overall survival.

Keywords:
Cedrella odorata L
Efficient light use
Efficient water use
Ochroma pyramidale (Cav. Ex Lam.)
Pachira quinata Jacq

RESUMEN

El Bosque seco tropical es uno de los ecosistemas más afectados en el mundo por el desarrollo de actividades antrópicas, por lo que es necesario estudiar las dinámicas de su ecosistema con el fin de restaurarlo. Con el objetivo de determinar la supervivencia, desarrollo y comportamiento fotosintético de las especies forestales en edades tempranas, se realizó un estudio de campo con tres especies: *Cedrela odorata* L. (cedro rojo), *Pachira quinata* (Jacq.) W.S. Alverson (ceiba tolúa*)* y *Ochroma pyramidale* (Cav. ex Lam.) Urb. (balso). Los datos de campo fueron recolectados en diferentes períodos, cuyas condiciones climáticas fueron: período seco (S.0), primer período lluvioso (Ll.1), primer período seco (S.1), segundo período lluvioso (Ll.2) y segundo período seco (S.2). Se midió la altura total (H) y el diámetro en la base (RCD), y se realizó dos cosechas para medir el peso seco. Además, se evaluó el funcionamiento fotosintético y su efecto en el desarrollo de las especies en tres periodos pluviométricos contrastantes midiendo la radiación fotosintéticamente activa (PAR), conductancia estomática (g_s), carbono intercelular (C_{int}), fotosíntesis neta (PN), transpiración (t_{rans}), uso eficiente del agua (EWU) y uso eficiente de la luz (ELU) entre las 8:00 y 17:00 h del día. Así mismo, se realizaron dos cosechas, para la medición del peso seco. Se realizó un análisis de varianza, encontrando diferencias significativas (P<0.05) en la interacción en H y RCD, y en la variable fotosintética PN. Las variables g_s y t_{rans} mostraron significancia estadística con las especies y los periodos pluviométricos; C_{int} fue significativa sólo en los periodos pluviométricos. *O. pyramidale* fue la especies que mayor supervivencia presentó y toleró las condiciones climáticas desarrollando mecanismos fisiológicos, mientras que *C. odorata* fue la especies más afectada en términos de supervivencia por las condiciones climáticas.

Palabras clave:
Cedrella odorata L
Uso eficiente de la luz
Uso eficiente del agua
Ochroma pyramidale (Cav. Ex Lam.)
Pachira quinata Jacq

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The dry tropical forest (TDF) consists of continuous forest cover and is found in regions with an average annual temperature higher than 27 °C and average annual precipitation of 1058 mm (Stoner and Sánchez-Azofeifa, 2009). The dry tropical forest is one of the ecosystems most threatened by anthropic activities because its soils are fertile and well-suited for agriculture (Calvo-Alvarado *et al.*, 2009; Quesada *et al.*, 2009). It is estimated that 48.5% of the world's TDF have been used for different purposes than its conservation (Portillo-Quintero and Sánchez-Azofeifa, 2010). These disturbances have given rise to plant distinct covers from those naturally found in TDF because natural regeneration processes do not ensure a return to the original state (Griscom and Ashtom, 2011). Nearly 1,000,000 km^2 of the world's remaining TDF is threatened by the expansion of human populations, habitat fragmentation, and climate change, and just 30% of TDFs are protected under conservation regimes (Fajardo *et al.*, 2013). Besides, 350 million ha have been deforested, and another 500 million ha of primary and secondary forest has been degraded (Lamb *et al.*, 2005). Restoration and replanting native species are strategies that can guarantee the continued provision of environmental goods and services, in addition to protecting and recovering native flora (Bastien-Henri *et al.*, 2010). These strategies have produced positive results, including the recovery of soils and nutrients and the establishment of plant cover and water balance in degraded areas (dos Santos *et al.*, 2006).

Nevertheless, one of the metrics governing whether a plantation or reforestation program is successful is the survival of plants species and its growth and development time because these characteristics depend mainly on the source of seeds, the environmental requirements, and microclimatic conditions (Allen *et al.*, 2010). The establishment of species and provenance trials has become a forestry tool that enables the development of programs aimed at genetic improvement for the propagation of species at determined sites, ensuring the quality of the conservation effort. At the same time, these trials provide information about species potential for use in the plant recovery of degraded areas.

Various experiments involving native species in tropical countries have been performed, and interest in this subject has grown due to the lack of information about which species will contribute most to the success of reforestation and restoration programs (Niinemets and Valladares, 2006). Despite the wide diversity of species found in tropical forests, commercial reforestation programs most commonly utilize exotic species. The most frequently used genera in the tropical Americas are *Tectona*, *Eucalyptus*, *Pinus*, and *Acacia* (Bastien-Henri *et al.*, 2010; van Breugel *et al.*, 2011). This common practice is because there is a risk to utilize new species when little is known about its management or its growth and survival rate under natural conditions (dos Santos *et al.*, 2006).

Species such as *Cedrela odorata, Pachira quinata*, and *Ochroma pyramidale*, which are native to the tropical Americas, are known for their high commercial value. However, little information is available regarding their development and adaptation to the climatic conditions of TDF, where seasonal variations are marked by intense dry periods alternated with rainy periods, the latter of which is decisive for the growth, phenology and photosynthetic response of the plants in TDF (Eamus, 1999).

Microclimatic factors such as temperature, water availability, and relative humidity can generate stress for plants that directly impacts their physiological and photosynthetic development (Marenco *et al.*, 2003; Briceño, 2017). For this reason, plants have developed diverse adaptation strategies; the results of which are manifested in growth, reproduction, survival, abundance, and geographical distribution (Cai *et al.*, 2009; Araque *et al.*, 2009; Esmail and Oelbermann, 2011).

With the progressive disappearance of TDF at a global level, it is necessary to obtain a better understanding of the effects of extreme climatic factors on the early establishment of native forest species and to identify the ecological requirements of these plants (Stoner and Sánchez-Azofeifa, 2009). The objective of the study was to advance the monitoring of three species in their survival, dasometric parameters, and gaseous exchange of foliage in TDF conditions in contrasting rainfall periods.

MATERIALS AND METHODS

Study area

This research was performed as part of the forest species test carried out in the project "*Study of the recovery of degraded areas in the dry tropical forest, Olaya municipality*." Founded by the Inter-administrative

Agreement 8787 of 2010, which included the Universidad Nacional de Colombia-Sede Medellín and the Corporación Autónoma Regional de Antioquia (Corantioquia). The study was developed at the Tribio Mamey ranch located in the Sucre township, Olaya municipality, Antioquia department (6°35´33.72"N, 75°47´33.70"W) (Colombian Andean region), between 540 and 680 m of altitude. The Sucre township registers an average annual rainfall of 1058 mm and an average temperature of 27.1 °C (registering minimum temperatures of 21 °C and maximum temperatures of 40.5 °C) which places it in the dry tropical forest life zone.

During the first half of the year, rainfall occurs in April and May, with the highest amount registered in April, and temperatures reaching 37 °C. The second rainy period occurs in September and October, with the highest levels of precipitation in October. The periods with dry tendencies in the first half of the year occur during January and February, which register the highest temperatures of the year (38 °C); in the second half of the year, the dry periods occur in June and July, with temperatures between 37 and 38 °C. In December, rainfall decreases, and the temperature begins to rise (IDEAM, 2013).

Species, characteristics of the plots and evaluated variables

Between March 2011 and February 2013, the inter-administrative study was carried out with 11 native species of the TDF (Table 1), in an area of 23.66 ha. From this species, *P. quinata, C. odorata*, and *O. pyramidale* were selected because they have a high potential to be used in reforestation and restoration processes, which were followed up on their gas exchange and dasometric characteristics**.** Each species was planted over an area of one hectare distributed randomly in four complete blocks of 2,500 m^2 at a planting density of 3×3 m (i.e., 1,100 tree ha^{-1}). At the beginning of the study, the land was cleared manually with a machete, and the trees and shrubs growing as a part of natural regeneration processes were left intact. A maintenance procedure consisting of clearing one-meter radius around each plant with a machete was performed every six months. A circular plot of 250 m^2 was established in the center of each block with an average of 28 specimens per plot; each specimen was identified and labeled. *C. odorata* seeds were provided by Corantioquia and were originally obtained in the Andean region of Colombia, while Balsur and Monterrey Forestal Ltda. companies provided the *O. pyramidale* and *P. quinata* seeds, respectively. Both came from Colombia's Atlantic region. Seed handling and the chosen pretreatments for optimal germination were based on standard recommendations for these species.

Table 1. Species used in the study Inter-Administrative Agreement 8787 of 2010[1].

Species		Family
Common name	**Scientific name**	
Yellow cedar	*Albizia guachapele*	Mimosaceae
Sandbox tree	*Hura crepitans*	Euphorbiaceae
Elephant-ear tree	*Enterolobium cyclocarpum*	Mimosaceae
Locust tree	*Hymenaea courbaril*	Caesalpiniaceae
Balsa	*Ochroma pyramidale*	Bombacaceae
Red ceiba	*Pachira quinata/Bombacopsis quinata*	Bombacaceae
Golden trumpet tree	*Tabebuia chrysantha*	Bignoniaceae
Spanish cedar	*Cedrela odorata*	Meliaceae
Coffeewood	*Caesalpinia ebano*	Caesalpiniaceae
Flamboyant	*Delonix regia*	Caesalpiniaceae
Gumbo limbo	*Bursera simaruba*	Burseraceae

[1] Technical report from the study project for the recovery of degraded areas in the dry tropical forest, Olaya municipality. Universidad Nacional de Colombia Sede Medellín and Autonomous Regional Corporation of Antioquia (2012).

Data were collected in the following periods, whose climatic conditions were: dry period S.0 (Marzo 2011), rainy period Ll.1 (Apri-May 2012), dry period S.1 (June-September 2012), rainy period Ll.2 (October-November 2012) and dry period S.2 (January-February 2013). The last period was not considered in the analysis of photosynthesis data due to the defoliation of the *C. odorata* and *P. quinata* species. During each of these periods, the species was 13 (Ll.1), 17 (S.1), 21 (Ll.2) and 23 (S.2) months old; where the total height (H) and the root collar diameter (RCD) were measured, repeatedly. A count of standing specimens was taken during each round of data collection to perform a survival analysis, and the percentage of survival for each rainfall period was determined (Ll.1, S.1, Ll.2, and S.2).

The following photosynthetic variables: photosynthetically active radiation, PAR (μmol m^{-2} s^{-1}), stomatal conductance, g_s (mmol m^{-2} s^{-1}), intercellular carbon, C_{int}, net photosynthesis, NP (μmol m^{-2} s^{-1}), and transpiration, t_{rans} (mmol m^{-2} s^{-1}), were measured simultaneously between 8:00 and 17:00 h (Ellis *et al*., 2000; Krause *et al*., 2001; Marenco *et al*., 2003; Juhrbandt *et al*., 2004; Araque *et al*., 2009). A healthy mature leaf in the upper third of the canopy was selected for each using an infrared gas analyzer (IRGA, TPS – 2 PPSYSTEMS). Efficient water use (EWU) and efficient light use (ELU) (Larcher, 1995; Lambers *et al*., 2008) were calculated using the equations:

$$EWU = \frac{NP}{trans}\left(\mu molCO_2 m^{-2} s^{-1} / mmolH_2O\, m^{-2} s^{-1}\right) \quad (1)$$

$$ELU = \frac{NP}{PAR}\left(\mu molCO_2 m^{-2} s^{-1} / \mu molphotons\, m^{-2} s^{-1}\right) \quad (2)$$

The total height (H) of each specimen was measured with a tape, measuring from the base of the tree to the terminal bud (in the cases for which this was not possible, height was measured up to the highest leaf) and the root collar diameter (RCD) was measured at the height of 5 cm from the ground.

For the collection of biomass data, two specimens were harvested manually from each plot: one at the beginning of data collection (April-May 2012) and the other at the end (January-February 2013). In the field, the specimens were divided into the stem, leaves, and roots (primary and secondary). The samples were incubated at 60 °C until they reached a constant weight to determine their dry weight (g) in the Ecology Laboratory - Biogeochemical Area of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia - Sede Medellín.

Statistical design

To determine the behavior of the dasometric and photosynthetic variables between rainfall periods (Ll.1, S.1, and Ll.2) and species, an analysis of variance was performed, adjusting the first-order autoregressive covariance structure (type=arh(1)) through the use of mixed models. In the factorial structure (Time×Treatment). Time represents each of the stages of the bimodal regime in which the measurements were taken, and Treatment represents each of the three species. Significant differences (P<0.05) were determined using the Fisher test (Least Significant Difference). The assumption of normality was confirmed using the Kolmogorov-Smirnov test (Pr>D). The statistical program SAS® 9.2 (SAS Institute Inc. 2004) was used.

RESULTS AND DISCUSSION

Survival and growth of the species in TDF climate conditions

During the first rainy period (Ll.1, 13 months old), the three species presented similar averages of survival (94 and 99%), *C. odorata* species registered the lowest values. During the final period, *C. odorata* registered the lowest survival rate (15%), *P. quinata* registered 42%, and *O. pyramidale* registered 55% (Figure 1). After thirteen months, the three species showed similar behaviors in Ll.1 with high levels of survival. *O. pyramidale* was the species that shows the highest percentage of survival during periods S.1 and Ll.2, identical to the findings of Craven *et al.* (2007) with the same species in a dry tropical forest. These results also confirm that *O. pyramidale* is species that easily acclimate to weather variation (Oberbauer and Strain, 1984; Kitajima, 1994; Krause *et al*., 2001). Besides, many perennial species growing in dry climates avoid the effects of drought conditions by developing a deep root system that allows them to capture water in soil zones that are sometimes close to the phreatic stratum (Castellanos and Newton, 2015*)*. Moreover, water deficiency is an important environmental limit that is related to the physiological processes involved in the growth and development of plants. It influences a set of responses to the sequence

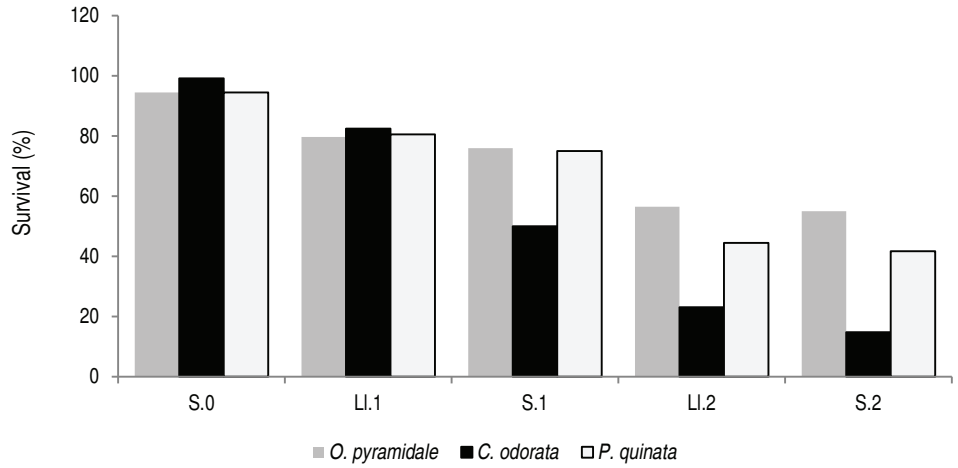

Figure 1. Survival (%) for three species during rainy period (Ll.1, April-May 2012), dry period (S.1, June-September 2012), rainy period (Ll.2, October-November 2012) and dry period (S.2, January-February 2013).

that mainly affects the mechanism of gas exchange (Centritto *et al*., 2009).

There were significant differences in the interaction time×species in height (P<0.0002) and diameter (P<0.0003). The species that registered the greatest height was *O. pyramidale*, which reached 180 cm during the final period (S.2), followed by *P. quinata* at 68.55 cm and *C. odorata* at 50.74 cm. Regarding diameter, at 23 months after planting (S.2), *O. pyramidale* had the greatest diameter of 3.43 cm, while *P. quinata* registered a diameter of 1.43 cm and *C. odorata* of 1.01 cm.

The accumulation of total biomass of the species under study was 1,787.87 g in *O. pyramidale*, 795.61 g for *P. quinata*, while *C. odorata* had a biomass of 222.60 g (Table 2).

O. pyramidale also presented greater growth both in diameter and in height, supporting the findings of Wishnie *et al.* (2007) in the dry zones of Panama, where they reported higher annual growth in height and diameter for *O. pyramidale* than for *C. odorata* and *P. quinata*. Similarly, during the Ll.1 rainy period, this species registered greater leaf production, which agrees with the findings of dos Santos *et al.* (2006), who reported that in conditions of high solar radiation, some species make optimal use of light energy, transforming it into ATP to increase their biomass.

Site conditions had a greater influence on the survival of *C. odorata*, and confirm the reported by Gerhardt (1998) because, under dry conditions with a controlled supply of water, this species increases its survival in the field. *C. odorata* also displayed low levels of survival in plantation trials performed on Ecuadorian pastureland, Davidson *et al.* (1998) reported low survival rates for *C. odorata* (less than 50%) in the dry zones; however, with water supply, the death of the species notably decreased. Similarly, Esmail and Oelberman (2011) reported that *C. odorata* seedlings that were constantly irrigated when exposed to high-temperature conditions (34 °C) exhibited an improvement in growth response regarding height and biomass.

Between the three species, it was observed that *C. odorata* showed total defoliation during the dry periods, in addition to low foliar mass production during the rainy periods. As a result, their accumulated total biomass was low during the study period. This behavior happens because the decrease in the foliar area is one of the strategies employed by plants to counteract the stress caused by a lack of water (Lambers *et al*., 2008). Along these lines, in Panama, Craven *et al.* (2007) found that

species such as *C. odorata* maintained low leaf area and growth levels, leading to the conclusion that these plants invested more energy into withstanding stressful conditions than into accumulating biomass.

Table 2. Height, diameter and biomass of the three species during S.0 dry period (March 2011), rainy period (LI.1, April-May 2012), dry period (S.1, June-September 2012), rainy period (LI.2, October-November 2012) and dry period (S.2, January-February 2013).

Species	Rainfall period	H (cm)	RCD (cm)	Growth rate		Biomass (g)		
				H (cm month^{-1})	RCD (cm month^{-1})	Root	Stem	Leaves
O. pyramidale	S.0	45.25 a	1.02 a	-	-	-	-	-
	LI.1	91.66 b	2.23 b	11.60	0.30	123.22	150.58	246.99
	S.1	136.75 c	3.01 c	11.27	0.20	-	-	-
	LI.2	175.08 d	3.47 d	9.58	0.12	-	-	-
	S.2	180.00 e	3.43 d	1.23	-0.01	495.61	579.50	191.97
C. odorata	S.0	43.16 a	0.64 a	-	-	-	-	-
	LI.1	46.66 a	1.05 a	0.88	0.10	21.95	22.00	9.67
	S.1	49.60 a	1.06 a	0.74	0.00	-	-	-
	LI.2	50.66 a	0.98 a	0.26	-0.02	-	-	-
	S.2	50.74 a	1.01 a	0.02	0.01	73.10	95.44	0.44
P. quinata	S.0	38.66 a	0.94 a	-	-	-	-	-
	LI.1	53.66 a	1.21 a	3.75	0.07	128.93	43.04	35.82
	S.1	63.66 a	1.27 ab	2.50	0.02	-	-	-
	LI.2	60.00 a	1.47 ac	-0.91	0.05	-	-	-
	S.2	68.55 a	1.43 a	2.14	-0.01	268.42	319.40	0

The rows with identical letters indicate no significant differences registered between rainfall periods.
H: total height, RCD: root collar diameter.

P. quinata species has been adapted to weather conditions, and it is resistant to low rainfall rates, which facilitated its establishment in this degraded area. In the dry regions, low mortality values have been reported for *P. quinata* (Hall *et al*., 2011). At the same time, Kane *et al*. (1993) noted that this species maintains considerable reserves of starch in its root system, which allow it to have a rapid initial growth at the beginning of the rainy season. Consistently, an increase in growth rate was observed during the LI.1 period. The strategy employed by the plant was the reduction of its leaf biomass during the dry periods to combat hydric stress. According to Eamus (1999), stomatal sensibility in caducipholic plants increases with soil dryness, and a result attributed to the decrease in elasticity of their cell walls that in turn results in a high propensity to the loss of turgidity. *P. quinata* specimens exhibited the greatest diameter, coinciding with the findings of Wishnie *et al.* (2007) in a study of 24 species with restoration potential and commercial value. *P. quinata* is a species that acclimate to conditions of high solar radiation and low humidity (Kane *et al*., 1993). Although soil fertility was not evaluated in the present research, it could be a factor that impacts its growth (Hall *et al*., 2011). *O. pyramidale* performed the best in terms of height due to the ease with which it acclimates (Krause *et al*., 2001). The results obtained are consistent with the findings of Wishnie *et al*. (2007) in Panama in a study of TDF species that also included *C. odorata* and *P. quinata*. These findings also confirm that *O. pyramidale* is a fast-growing species that acclimate well to dry areas.

Photosynthetic behavior of the species studied

It must be considered the importance of the leaves does not exclusively lie in carrying out the photosynthetic process. They are involved in nutrient storage and photoassimilate process, and as sources of nutrients in the processes of metabolic remodeling during organ senescence (Severino and Auld, 2013). In their early stages, the seedlings developed physiological mechanisms that allowed them to increase CO_2 assimilation during the dry period. Water deficiency is an important environmental limitation that affects all the physiological processes involved in the growth and development of plants. It influences a set of responses to the drought that mainly affects the mechanism of gas exchange (Centritto *et al.*, 2009). The stomatal regulation of transpiration and intercellular carbon concentration during dry periods did not decrease the CO_2 assimilation rate; for optimal control of stomata to manage hydraulic risk is likely to have significant consequences for ecosystem fluxes during drought, which is critical given projected intensification of the global hydrological cycle (Anderegg *et al.*, 2018).

The foliage response of these three species had a relationship with the climate. In such a way that stomatal regulation was identified as the rainfall was presented with stomata partially open until almost closed depending on the species. Thus, during the rainy periods, *O. pyramidale* and *C.odorata* showed partially open stomata, regardless of whether they were, although in a different degree of openness. On the contrary, *P.quinata* remained with the stomata partly or almost closed in the two climatic seasons (Table 3).

Table 3. Foliage response to the gaseous exchange of the three species during dry period (S.0, March 2011), rainy period (LI.1, April-May 2012), dry period (S.1, June-September 2012), rainy period (LI.2, October-November 2012) and dry period (S.2, January-February 2013).

Rainfall period	Species	Age (month)	PAR (µmol Phot m^{-2} s^{-1})	g_s (mmol H_2O m^{-2} s^{-1})	NP (µmol CO_2 m^{-2} s^{-1})	C_{int}	t_{rans} (mmol H_2O m^{-2} $s^{-1)}$	EWU (µmol CO_2 $mmol^{-1}$ H_2O)
LI.1		13	1,150.90 a	281.89 b	11.95 a	304.17	2.38	5.25
S.1	*O. pyramidale*	17	2,168.83 b	54.50 ab	24.34 b	0.10	0.94	14.93
LI.2		21	682.25 a	25.83 a	7.68 a	71.00	0.29	4.06
LI.1		13	1,997.33 a	569.58 a	16.83 a	244.00	3.68	4.62
S.1	*C. odorata*	17	1,466.24 ba	35.29 b	11.02 a	34.00	0.98	14.66
LI.2		21	835.60 b	342.19 a	16.09 a	107.00	2.25	3.29
LI.1		13	2,401.17 a	59.16 a	12.90 a	147.17	1.99	8.60
S.1	*P. quinata*	17	1,799.25 a	2.16 a	9.96 a	3.60	0.09	10.94
LI.2		21	580.42 b	1.50 a	11.24 a	31.00	0.068	29.34

The rows with identical letters indicate no significant differences registered between rainfall periods.
PAR: Photosynthetically active radiation, g_s: stomatal conductance, NP: net photosynthesis, C_{int}: intercellular carbon, t_{rans}: transpiration, EWU: efficient water use, ELU: efficient light use.

According to Berry *et al.* (2010), plants with better control of stomatal function are more efficient in the use of water and have more tolerance to the drought. It is indicative that stomatal control is an important adaptive mechanism of tolerance to the drought in this species (dos Santos *et al.*, 2017). The partial closure of the stomata is a known strategy of plant tolerance to water stress since it decreases the rate of transpiration, conserves the water content of the leaves, and reduces the risk of dehydration avoiding death by desiccation (Peak *et al.*, 2004).

The vapor pressure deficit (VPD) is one of the most important environmental factors of stomatal regulation since plants from semi-arid regions showed an inverse correlation between leaf with stomatal conductance, transpiration, and photosynthesis (dos Santos *et al.*, 2017). Likewise, the castor has a high stomatal

regulation under field conditions, which can reduce the loss of water by transpiration and maintain the hydric state of the plant (Pinheiro and Chaves, 2011).

The previous response of the species under study represented that *O. pyramidale* exhibited the highest NP in the dry period (24.31 µmol CO_2 m^{-2} s^{-1}), with the lowest average NP in the rainy period (9.81 µmol CO_2 m^{-2} s^{-1}). *C. odorata* expressed the highest average photosynthetic rate (16.43 µmol CO_2 m^{-2} s^{-1}) in rainy periods and intermediate in dry periods (11.02 µmol CO_2 m^{-2} s^{-1}), and *P. quinata* the lowest NP in the two climatic conditions. It can be affirmed that these species are from the group of C_3 plants by considering the photosynthetic rates. According to Ocheltree *et al.* (2014), C_3 plants reduce stomatal conductance to minimize water loss; however, the rate of CO_2 diffusion also decreases, which reduces the internal concentration of CO_2 and the efficiency in carbon fixation by plants (Table 3).

Stomatal regulation of the transpiratory process was observed in these species that are adapted to arid and semi-arid regions, with average rates of 2.96 mmol m^{-2} s^{-1} in the rainy period and 0.98 mmol m^{-2} s^{-1} in the dry period for *C. odorata*; 1.33 and 0.94 mmol m^{-2} s^{-1} in *O. pyramidale* and in *P. quinata* 1.03 and 0.09 mmol m^{-2} s^{-1} during the same climatic periods. It led to divergences between the species studied since *P. quinata* expressed average EWU values of 12.64 µmol CO_2 $mmol^{-1}$ H_2O in the rainy season, but in the dry period, it was 10.94 µmol CO_2 $mmol^{-1}$ H_2O. On the other hand, both *O. pyramidale* and *C. odorata* were around 3.00 and 14.00 µmol CO_2 $mmol^{-1}$ H_2O in the rainy and dry periods respectively.

In different species, including soybean, it has been found that the reduction in g_s increases the intrinsic efficiency of water use, especially with little availability of water in the soil (Gilbert *et al*., 2011). Barros Junior *et al.* (2008) found that the castor, under drought stress, showed a high efficiency in the use of water, which helped maintain the production of biomass.

The reduction in stomatal conductance has been correlated with an increase in the intrinsic efficiency of water use, which indicates that the closure of stomata contributes to optimizing the efficiency of water use in plants under stress. It allows plants to absorb carbon by decreasing the loss of water in the hottest part of the day, contributing to the maintenance of photosynthesis (Broeckx *et al*., 2014). It can be considered that EWU is a preventive mechanism, as an immediate effect of water deficiency. Besides, from the physiological point of view, the high value of EWU is traditionally considered as a mechanism that provides greater productivity and survival in dry environments (Centritto *et al*., 2009; Gilbert *et al*., 2011).

Under conditions of water stress, the castor plant maintains an effective stomatal regulation with a high net CO_2 fixation (Severino *et al*., 2012); with a decrease in perspiration due to rapid stomatal closure, without damage to the photosynthetic apparatus because the deficiencies in the fixation and capture of C are due to the diffusive resistances (Sausen and Rosa, 2010). For this property, they can partially recover the functioning of the photosynthetic apparatus, while remaining in stress; but, when this is eliminated, the plants recover their photosynthetic function in 24 h (Severino *et al*., 2012). Consequently, they tolerate drought stress quite well; they become a viable crop for arid and semi-arid regions where there are few effective agricultural alternatives (Sausen and Rosa, 2010).

However, this research information is scarce in field conditions to better understand the physiological mechanisms and their interactions with climatic factors under drought (dos Santos *et al*., 2017). Finally, Anderegg *et al.* (2018) found that the stomatal response to environmental conditions forms the backbone of all ecosystem models and carbon cycles; but relies heavily on empirical relationships. Evolutionary theories of stomatal behavior are critical to protecting against prediction errors of empirical models in future climates. A longstanding theory holds that stomata maximize the ability to maintain a constant marginal efficient water use over a given time horizon. However, a recent evolutionary theory proposes that stomata instead of maximizing carbon gain reduce carbon costs/risk of hydraulic damage. Anderegg's *et al.* (2018) findings focus on the constant known as "marginal efficiency of water use" when it is not the quantity of water that governs the evolution of stomatal regulation, but the stomatal regulation is maximized with the carbon gain while maintaining the hydraulic function.

CONCLUSIONS

There were significant differences in the interaction time×species regarding height (P<0.0002) and diameter (P<0.0003), the highest was *O. pyramidale,* followed by *P. quinata* and *C. odorata*. In regard to diameter, *O. pyramidale* had the greatest diameter after 23 months (S.2) and biomass accumulation, followed by *P. quinata* and *C. odorata*.

Foliage response of these three species had a relationship with weather conditions, during the rainy periods *O. pyramidale* and *C.odorata* showed partially open stomata regardless of whether they were. Besides, *P.quinata* remained with the stomata partly or almost closed in the two climatic seasons.

The behavior of the stomatal regulation was detected in species adapted to arid and semi-arid conditions, in such a way, the intensity of the photosynthetic and transpiratory rates and the efficient water use were expressed according to the genotype×environment interaction. However, it would be interesting to auscultate and use the EWU to identify the stomatal regulation with the carbon cost/risk of hydraulic damage.

REFERENCES

Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, Vennetier M, Kitzberger T, Rigling A, Breshears DD, Hogg EH, Gonzalez P, Fensham R, Zhang Z, Castro J, Demidova N, Lim JH, Allard G, Running SW, Semerci A and Cobb N. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. Forest Ecology and Management 259(4): 660–684. doi: 10.1016/j.foreco.2009.09.001

Anderegg WRL, Wolf A, Arango A, Choat B, Chmura DJ, Jansen S, Kolb T, Li S, Meinzer FC, Pita P, Resco de Dios V, Sperry JS, Wolfe BT and Palaca S. 2018. Woody plants optimise stomatal behaviour relative tohydraulic risk. Ecology Letters 21(7): 968–977. doi: 10.1111/ele.12962

Araque O, Jaimez RE, Azócar C, Espinoza W and Tezara W. 2009. Relaciones entre anatomía foliar, intercambio de gases y crecimiento en juveniles de cuatro especies forestales. Interciencia 34(10): 725-729

Barros Junior G, Guerra HOC, Cavalcanti MLF and de Lacerda RD. 2008. Consumo de água e eficiência do uso para duas cultivares de mamona submetidas a estresse hídrico. Revista Brasileira de Engenharia Agrícola e Ambiental 12(4) 350-355. doi: 10.1590/S1415-43662008000400003

Bastien-Henri S, Park A, Ashton M and Messier C. 2010. Biomass distribution among tropical tree species grown under differing regional climates. Forest Ecology and Management 260(3): 403-410. doi: 10.1016/j.foreco.2010.04.035

Berry JA, Beerling DJ and Franks PJ. 2010. Stomata: key players in the earth system, past and present. Current Opinion in Plant Biology 13(3): 232-239. doi: 10.1016/j.pbi.2010.04.013

Briceño A. 2017. Dinámica del crecimiento y relación con el clima de especies arbóreas de los bosques de la región Caribe, Colombia. Ph.D's Thesis in Science. Biology. Sciences Faculty. Universidad Nacional de Colombia. Bogotá. 157 p.

Broeckx LS, Fichot R, Verlinden MS and Ceulemans R. 2014. Seasonal variations in photosynthesis, intrinsic water-use efficiency and stable isotope composition of poplar leaves in a short-rotation plantation. Tree Physiology 34 (7): 701-715. doi: 10.1093/treephys/tpu057

Cai ZQ, Wang WH, Yang J and Cai CT. 2009. Growth, photosynthesis and root reserpine concentrations of two *Rauvolfia* species in response to a light gradient. Industrial Crops and Productos. 30(2): 220-226. doi: 10.1016/j.indcrop.2009.03.010

Calvo J, McLennan B, Sanchez A and Garvin T. 2009. Deforestation and forest restoration in Guanacaste, Costa Rica: Putting conservation policies in context. Forest Ecology and Management 258 (6): 931-940. doi: 10.1016/j.foreco.2008.10.035

Castellanos C and Newton AC. 2015. Grupos funcionales y variación en rasgos foliares y de tallo de 113 especies leñosas en un bosque seco tropical species . Colombia Forestal 18(1): 117-138. doi: 10.14483/udistrital.jour.colomb.for.2015.1.a07

Centritto M, Lauteri MC, Monteverdi MC and Serraj R. 2009. Leaf gas exchange, carbon isotope discrimination, and grain yield in contrasting rice genotypes subjected to water deficits during the reproductive stage. Journal of Experimental Botany 60(8): 2325-2339. doi: 10.1093/jxb/erp123

Craven D, Braden D, Ashton MS, Berlyn GP, Wishnie M and Dent D. 2007. Between and within-site comparisons of structural and physiological

characteristics and foliar nutrient content of 14 tree species at a wet, fertile site and a dry, infertile site in Panama. Forest Ecology and Management 238(1-3): 335-346. doi: 10.1016/j.foreco.2006.10.030

Davidson R, Gagnon D, Mauffette Y and Hernandez H. 1998. Early survival, growth and foliar nutrients in native Ecuadorian trees planted on degraded volcanic soil. Forest Ecology and Management. 105(1-3): 1-19. doi: 10.1016/S0378-1127(97)00295-8.

dos Santos UM, de Carvalho JF and Feldpausch TR. 2006. Growth, leaf nutrient concentration and photosynthetic nutrient use efficiency in tropical tree species planted in degraded areas in central Amazonia. Forest Ecology and Management 226(1-3): 299-309. doi: 10.1016/j.foreco.2006.01.042

dos Santos CM, Endres L, Ferreira VM, Silva JV, Rolim EV and Wanderley HCL. 2017. Photosynthetic capacity and water use efficiency *in Ricinus communis* (L.) under drought stress in semi-humid and semi-arid areas. Anais da Academia Brasileira de Ciências 89(4) 3015-3029. doi: 10.1590/0001-3765201720160729.

Eamus D. 1999. Ecophysiological traits of deciduous and evergreen woody species in the seasonally dry tropics. Trends in Ecology & Evolution 14 (1): 11-16. doi: 10.1016/S0169-5347(98)01532-8

Ellis A, Hubbell S and Potvin C. 2000. *In situ* field measurements of photosynthetic rates of tropical tree species: a test of the functional group hypothesis. Canadian Journal of Botany 78 (10): 1336-1347. doi: 10.1139/b00-095

Esmail S and Oelbermann M. 2011. The impact of climate change on the growth of tropical agroforestry tree seedlings. Agroforest Systems 83(2): 235-244. doi: 10.1007/s10457-011-9424-1

Fajardo L, Rodríguez JP, González V and Briceño JM. 2013. Restoration of a degraded tropical dry forest in Macanao, Venezuela. Journal of Arid Environments (88): 236-243. doi: 10.1016/j.jaridenv.2012.08.009.

Gerhardt K. 1998. Leaf defoliation of tropical dry forest tree seedlings – implications for survival and growth. Trees 13(2): 88-95. doi: 10.1007/PL00009741

Gilbert ME, Zwieniecki MA and Holbrook NM. 2011. Independent variation in photosynthetic capacity and stomatal conductance leads to differences in intrinsic water use efficiency in 11 soybean genotypes before and during mild drought. Journal of Experimental Botany 62(8): 2875-2887. doi: 10.1093/jxb/erq461.

Griscom HP and Ashton MS. 2011. Restoration of dry tropical forests in Central America: A review of pattern and process. Forest Ecology and Management 261(10): 1564-1579. doi: 10.1016/j.foreco.2010.08.027

Hall JS, Ashton MS, Garen EJ and Shibu J. 2011. The ecology and ecosystem services of native trees: Implications for reforestation and land restoration in Mesoamerica. Forest Ecology and Management 261(10): 1553-1557. doi: 10.1016/j.foreco.2010.12.011

IDEAM. 2013. Boletín informativo sobre el monitoreo de los fenómenos de "el niño" y "la niña" 2013. In: Instituto de Hidrología, Meteorología y Estudios Ambientales, p.; accessed: May, 2013.

Juhrbandt J, Leuschner C and Hölscher D. 2004. The relationship between maximal stomatal conductance and leaf traits in eight Southeast Asian early successional tree species. Forest Ecology and Management 202(1-3): 245-256. doi: 10.1016/j.foreco.2004.07.021

Kane M, Urueña H, Dvorak W and Atehortua C. 1993. The potential of *Bombacopsis quinata* as a commercial plantation species. Forest Ecology and Management 56(1-4): 99-112. doi: 10.1016/0378-1127(93)90106-W

Kitajima K. 1994. Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. Oecologia 98(3): 419-428. doi: 10.1007/BF00324232

Krause GH, Koroleva OY, Dalling JW and Winter K. 2001. Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. Plant, Cell & Environment 24(12): 1345-1352. doi: 10.1046/j.0016-8025.2001.00786.x

Lamb D, Erskine PD and Parrotta JA. 2005. Restoration of degraded tropical forest landscapes. Science 310(5754): 1628-1632. doi: 10.1126/science.1111773

Lambers H, Chapin III FS and Pons TL. 2008. Plant Physiological Ecology. Second Edition. Springer Science+Business Media, New York. 604 p.

Larcher W. 1995. Physiological Plant Ecology: Ecophysiology and stress physiology of functional groups. Third Edition. Springer Science+Business Media, New York. 514 p.

Marenco RA, Vieira G and de Goncalves JF. 2003. Leaf micronutrient concentrations and potential photosynthesis in *Ochroma pyramidale* established in a degraded land. Acta Amazonica, 33(1):23-31. doi: 10.1590/1809-4392200331031.

Niinemets U and Valladares F. 2006. Tolerance to shade, drought, and waterlogging of temperate northern hemisphere trees and shrubs. Ecological Monographs 76(4): 521-547 doi: 10.1890/0012-9615(2006)076[0521:TTSDAW]2.0.CO;2

Oberbauer SF and Strain BR. 1984. Photosynthesis and successional status of Costa Rican rain forest trees. Photosynthesis Research 5(3): 227-232. doi: 10.1007/BF00030022.

Ocheltree TW, Nippert JB and Prasad PVV. 2014. Stomatal responses to changes in vapor pressure deficit reflect tissue-specific differences in hydraulic conductance. Plant, Cell & Environment 37(1): 132–139. doi: 10.1111/pce.12137

Peak D, West JD, Messinger SM and Mott KA. 2004. Evidence for complex, collective dynamics and emergent, distributed computation in plants. Proceedings of the National Academy of Sciences of the United States of America 101(4) 918-922. doi: 10.1073/pnas.0307811100

Pinheiro C and Chaves MM. 2011. Photosynthesis and drought: can we make metabolic connections from available data. Journal of Experimental Botany 62(3): 869-862. doi: 10.1093/jxb/erq340

Portillo CA and Sánchez GA. 2010. Extent and conservation of tropical dry forests in the Americas. Biological Conservation 143(1): 144-155. doi: 10.1016/j.biocon.2009.09.020

Quesada M, Sanchez A, Alvarez M, Stoner KE, Avila L, Calvo J, Casitllo A, Espíritu MM, Fagundes M, Fernades GW, Gamon J, Lopezaraiza M, Lawrence D, Cerdeira LP, Powers JS, Neves FS, Rosas V, Sayago R and Sanchez G. 2009. Succession and management of tropical dry forests in the Americas: Review and new perspectives. Forest Ecology and Management 258(6): 1014-1024. doi: 10.1016/j.foreco.2009.06.023

Sausen TL and Rosa LMG. 2010. Growth and carbon assimilation limitations in *Ricinus communis* (Euphorbiaceae) under soil water stress conditions. Acta Botánica Brasilica 24(3): 648-654. doi: 10.1590/S0102-33062010000300008

Severino LS, Auld DL, Baldanzi M, Cândido MJD, Chen G, Crosby W, He X, Lakshmamma P, Lavanya C, Machado OL, Mielke T, Milani M, Miller TD, Morris JB, Morse SA, Navas AA, Soares DJ, Sofiatti V, Wang ML, Zanotto MD and Zieler H. 2012. A Review on the Challenges for Increased Production of Castor. Agronomy journal 104(4): 853–880. doi: 10.2134/agronj2011.0210

Severino LS and Auld DL. 2013. A framework for the study of the growth and development of castor plan. Industrial Crops & Products 46: 25-38. doi: 10.1016/j.indcrop.2013.01.006.

Stoner KE and Sánchez GA. 2009. Ecology and regeneration of tropical dry forests in the Americas: Implications for management. Forest Ecology and Management (258): 903-906. doi: 10.1016/j.foreco.2009.05.019

van Breugel M, Hall JS, Craven DJ, Gregoire TG, Park A, Dent DH, Wishnie MH, Mariscal E, Deago J, Ibarra D, Cedeño N and Ashton MS. 2011. Early growth and survival of 49 tropical tree species across sites differing in soil fertility and rainfall in Panama. Forest Ecology and Management 261(10): 1580-1589. doi: 10.1016/j.foreco.2010.08.019

Wishnie MH, Dent DH, Mariscal E, Deago J, Cedeno N, Ibarra D and Ashton S. 2007. Initial performance and reforestation potential of 24 tropical tree species planted across a precipitation gradient in the republic of Panama. Forest Ecology and Management 243(1): 39-49. doi: 10.1016/j.foreco.2007.02.001.

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Cryoprotective effect of sorbitol on the muscle microstructure of yamú (*Brycon amazonicus*) during storage at 2 and -18 °C

Efecto crioprotector del sorbitol en la microestructura del músculo del yamú (*Brycon amazonicus*) durante su almacenamiento a 2 y -18 °C

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ABSTRACT

Although freezing is generally used to preserve the sensory and nutritional quality of fish and their products, it cannot mitigate physicochemical changes of the fish meat during storage. This study aimed to determine the cryoprotective effect of sorbitol incorporated into the yamú muscle (*Brycon amazonicus*), subjected to different storage times and temperatures. The methodology consisted of analyzing microstructural changes, protein profile, and physicochemical properties (texture, water holding capacity and pH) of the yamú's meat under two temperatures (2±2 and -18±2 °C), two storage times (24 and 48 h) and the incorporation or not of 5% (w/w) of a 60% sorbitol solution. The microstructural changes were analyzed by optical microscopy and scanning electron microscopy, and the protein profile was analyzed by SDS PAGE electrophoresis. The physicochemical properties evaluated in yamú's meat were affected mainly by the interaction between temperature and storage time. The myofibrillar proteins underwent a partial degradation, and changes in the connective tissue were observed concerning the loss of texture especially when the meat was not treated with sorbitol at freezing temperature (-18 °C). The use of sorbitol minimized the negative effects of freezing on the characteristics of the yamú muscle, maintaining the integrity of the muscular microstructure and generating a cryoprotective effect in comparison to untreated meat.

Keywords:
Cryoprotectant
Fish
Freezing
Myofibrillar proteins
Preservatives
Texture

RESUMEN

Aunque la congelación es generalmente usada para preservar la calidad sensorial y nutricional del pescado y sus productos derivados, no permite mitigar los cambios fisicoquímicos de la carne del pescado durante su almacenamiento. Este estudio tuvo como objetivo determinar el efecto crioprotector del sorbitol incorporado al músculo de yamú (*Brycon amazonicus*), sometido a diferentes tiempos y temperaturas de almacenamiento. La metodología consistió en analizar cambios microestructurales, perfil proteico y propiedades fisicoquímicas (textura, capacidad de retención de agua y pH) de la carne bajo dos temperaturas (2±2 o -18±2 °C), dos tiempos de almacenamiento (24 y 48 h) y la incorporación o no de 5% (p/p) de una solución de sorbitol al 60%. Los cambios microestructurales fueron analizados por medio de microscopia óptica y electrónica de barrido, el perfil proteico se analizó por medio de electroforesis SDS PAGE. Las propiedades fisicoquímicas evaluadas en la carne de yamú se vieron afectadas principalmente por la interacción entre la temperatura y el tiempo de almacenamiento. Las proteínas miofibrilares sufrieron una degradación parcial y se evidenciaron cambios en el tejido conectivo, relacionados con la perdida de textura especialmente cuando la carne no fue tratada con sorbitol a temperatura de congelación (-18 °C). El uso de sorbitol minimizó los efectos negativos de la congelación sobre las características del músculo de yamú, manteniendo en un estado más integro de la microestructura muscular, generando un efecto crioprotector en comparación con la carne no tratada.

Palabras clave:
Crioprotector
Pescado
Congelamiento
Proteínas microfibrilares
Conservantes
Textura

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Freezing is generally used to preserve the sensory and nutritional quality of fish and their products (Gonçalves *et al*., 2012). However, it cannot mitigate physicochemical changes during storage (Benjakul and Visessanguan, 2011) due to a different freezing speed between the outside and the inside of the cell, the migration of moisture from the interior of the cell to the extracellular space is generated, resulting in the growth of ice crystals (Hunt and Park, 2014). The formation of ice crystals and temperature changes during frozen storage damage the structure of the meat, which causes alterations in the biochemical reactions that occur at the cellular level and affect its physical quality parameters (Velasco *et al*., 2010; Benjakul and Visessanguan, 2011; Leygonie *et al*., 2012; Lee *et al*., 2017). These alterations, including the denaturation of the myofibrillar proteins, are associated with the loss of functional, nutritional and sensory properties of the meat, including gelling, emulsification, viscosity, solubility, and water holding capacity (Jacobsen *et al*., 2010; Andersen and Jørgensen, 2004; Lund and Baron, 2010; Nikoo *et al*., 2016).

Myofibrillar proteins include contractile proteins such as myosin and actin, regulatory proteins such as tropomyosin and troponin, and other minor proteins (Harnedy and Fitzgerald, 2012). These are mainly responsible for the functional properties of meat, especially myosin (Watabe *et al*., 1992; Ramírez *et al*., 2000) and are prone to denaturation during long-term frozen storage (Goeller *et al*., 2004; Medina and Pazos, 2010). Changes that occur in fish muscle during frozen storage can be minimized using appropriate cryoprotective additives (Nikoo and Benjakul, 2015), which can protect tissue from freeze damage by mitigating the growth of ice crystals (Alvarez *et al*., 2010). It is known that cryoprotectants such as carbohydrates and polyols reduce denaturation of myofibrillar protein during frozen storage, maintaining functional properties in meat (Kittiphattanabawon *et al*., 2012; Nikoo *et al*., 2016).

The yamú (*Brycon amazonicus*) is the most common species among the bryconids, native from the eastern Colombian plains, and the most explored for fish farming due to its omnivorous feeding habit, its rapid growth and nutritional efficiency, optimum taste of its meat, and its special characteristics for sport fishing (Arias, 2006). Yamú muscle is highly susceptible to quality loss induced during frozen storage. However, there are no studies on the effect of cryoprotectants on the fish's meat during frozen storage. For that reason, this study aimed to determine the cryoprotective capacity of sorbitol when it is incorporated into the yamú muscle and subjected to different storage times and temperatures.

MATERIALS AND METHODS

Biological material

The yamú fish (*Brycon amazonicus*) were obtained from an aquaculture production farm located in the Municipality of Lejanías, Meta, Colombia (3°31'33.8"N, 74°01'20.9"W), with a variable temperature from 6 °C in the paramo to average temperatures of more than 24 °C in the plain. 24 specimens of approximately 500 g each, fed with an artisanal diet, were sacrificed by thermal shock in ice water, eviscerated and immediately sent to the laboratory, kept packed in plastic bags under refrigeration at 4 °C in polystyrene refrigerators with ice for 4 h. Upon arrival, the specimens were washed with cold water (5±1.5 °C), and two fillets were obtained from each one.

Experimental treatments and sample preparation

The fillets were stored at 2±2 °C and -18±2 °C, for 24 and 48 h with the injection of a 60% sorbitol solution (Sorbitol USP 70%, Ciacomeq SAS) until achieving an absorption of 5% (w/w). In total, eight treatments with three repetitions each were performed (Table 1).

Texture analysis

The texture was evaluated by a compression test using an electronic texturometer model Stable Micro System texture analyzer (TA.XT2, Surrey, England) (Larsson *et al*., 2014). All tests were done at refrigeration temperature (4 °C). Three cubes of 2 cm×2 cm×1 cm were taken from each fillet – the speed before the test: 1.00 mm s^{-1}, the speed of the test: 1.10 mm s^{-1} and the speed after the test: 10.00 mm s^{-1}, the distance between the cylinder and the sample: 15.0 mm, and the sample compression: 40.0%.

Water holding capacity (WHC)

The water holding capacity (WHC) of the yamú meat samples was carried out following the methodology proposed by Sánchez-Alonso *et al.* (2012). Three grams of the sample were wrapped in filter paper (two filter papers

Whatman No. 1, 110 mm diameter) previously weighed, then they were introduced in a falcon tube and centrifuged for 15 min at 3000 g. After centrifugation, the papers were carefully removed and weighed. The WHC was expressed as a percentage of water retained by the sample after centrifugation.

Table 1. Experimental design of the yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage.

Treatment	1	2	3	4	5	6	7	8
Sorbitol injection	without sorbitol	with sorbitol	without sorbitol	with sorbitol	without sorbitol	with sorbitol	without sorbitol	with sorbitol
Temperature	2±2 °C		-18±2 °C		2±2 °C		-18±2 °C	
Storage time	24 h				48 h			

pH measurement

The pH of the resulting suspension was measured with a calibrated pH meter after homogenizing 10 g of the sample with 100 mL of distilled water according to the methodology described by Mohan *et al.* (2007).

Optical microscopy

Samples were immersed in formalin buffer at 4% and embedded in paraffin. The plates with the extended sample were stained with Masson's trichrome in order to highlight the collagen fibers. For this process, they were initially washed with distilled water and then immersed in Weigert's Hematoxylin for 5 min, then washed with distilled water and immersed in Ponceau fuchsin for 5 min, then immersed in phosphomolybdic acid for 5 min. This solution was transferred to a blue aniline solution for 5 min. Finally, the samples were analyzed with a microscope (Leica DM750 P, Leica Microsystems, Heerbrugg, Switzerland) and images were taken with different approaches (4X, 10X and 40X) (Castañeda *et al*., 2016).

Scanning electron microscopy (SEM)

The samples for this procedure were immersed in formalin buffer at 4%, then dried to critical point using the EK 3150 equipment for 15 min and then metalized with gold using an equipment Quorum Q150R ES, to proceed to the observation with a microscope SEM (FEI, Quanta 200 -r). The fish samples were mounted on individual supports. The images obtained were captured with magnitudes of 200X, 1000X and 4000X (Castañeda *et al*., 2016).

Protein extraction and quantification

Protein extraction was carried out by applying modifications to the method proposed by Cao *et al*. (2006). 1 g of sample was homogenized in an Ultra-turrax® IKA T-25 USA homogenizer with four volumes of distilled-deionized water for 30 s and centrifuged at 5000 g and 4 °C for 5 min, the supernatant was removed. The precipitate was washed with 50 mM sodium phosphate buffer (pH 7.5) for 30 s; then the homogenate was centrifuged at 5000 g and 4 °C for 5 min, the precipitate was suspended in 4 volumes of cold sodium phosphate buffer 50 mM (pH 7.5), the centrifugation process was repeated twice, the supernatant was discarded, and one last centrifugation was performed at 3000 g for 15 min. The final precipitate was diluted with 50 mM phosphate buffer and 500 mM NaCl pH 8.0. Each of the protein extracts was added with 5 µL 100 mM iodacetamide and 25 µL Ethylenediaminetetraacetic acid (EDTA) 50 mM as enzyme inhibitors.

Quantification of each protein extract was carried out to standardize the amount of protein in each sample. Quantification of protein concentration was performed following the bicinchoninic acid (BCA) method, using a Pierce ™ BCA® Thermo Scientific protein determination kit for total protein counting. This method combines the protein reduction of cupric (Cu^{+2}) to cuprous cations (Cu^{+1}) in an alkaline medium and the reaction of green to purple color by the chelation of the BCA molecules with the cuprous ions. This water-soluble complex exhibits a strong absorbance at 562 nm that is almost linear with increasing protein concentrations in a range from 20 to 2000 µg mL^{-1}.

Electrophoretic mobility profiles by SDS-PAGE

For the identification of myofibrillar protein profiles of yamú meat, polyacrylamide gel electrophoresis was carried out with an electrophoresis system (Mini-PROTEAN® Tetra Vertical Electrophoresis Cell, Bio-Rad, 25 USA). The separation gel was 7%, the concentration gel was 5% (w/v), the run buffer used was Tris-glycine pH 8.3. 30 µg of total protein of the protein extracts obtained from each sample were used. The separation was performed at a constant voltage of 120 V for the concentration gel and 100 V for the resolution gel. A molecular weight standard from 12 to 225 kDa was used, and the staining was performed with colloidal blue G-250 (CBB), which was prepared by diluting 125 g of CBB in 125 mL of isopropanol, then 50 mL of glacial acetic acid was added, and a volume of 500 mL was completed with deionized distilled water (Laemmli, 1970).

Statistical analysis

A descriptive analysis was made by treatment to the data obtained for texture, water retention capacity, and pH, to explore measures of central tendency, dispersion, and atypical observations in the data. The eight treatments were analyzed according to a completely randomized design in a factorial arrangement 2^3, where the factors were storage temperature, time and use of sorbitol. Each treatment had three repetitions. An ANOVA was performed to determine whether there was a significant difference among the levels of each of the factors. When a difference was found between the levels of the factors, a Tukey test was performed to find which of the treatments had a significant difference. An ANOVA was performed for each response to establish the difference and interaction among factors. In the presence of interaction, the interaction graphs were constructed to be able to conclude on the best treatments. The R Studio 1.1.383® program (2017) was used.

RESULTS AND DISCUSSION

Texture analysis

The variance analysis performed for the texture parameters obtained indicate that there was a significant difference between treatments (P<0.05). The Tukey test suggests that the treatments subjected to the incorporation of sorbitol -18 °C for 24 and 48 h (T4 and T8) obtained the highest compression force value and did not present significant difference among them (Figure 1).

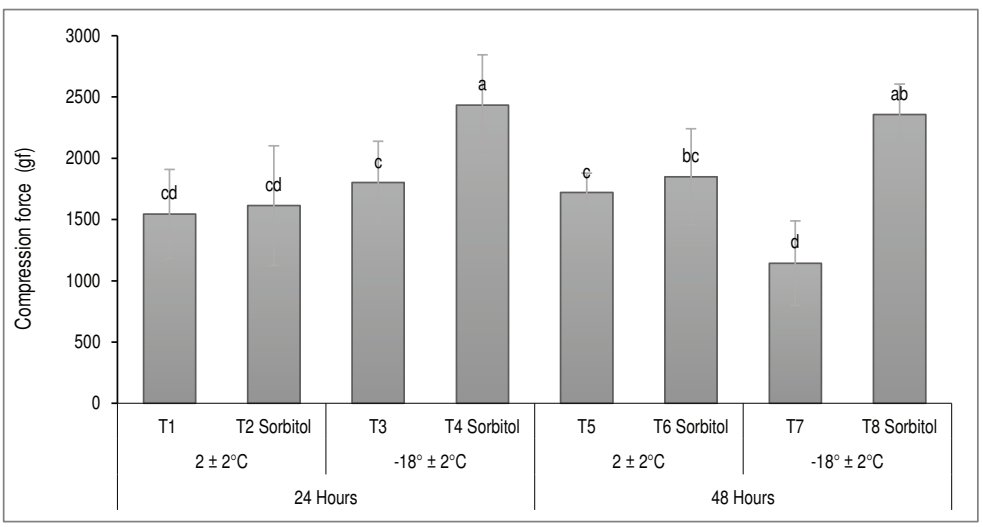

Figure 1. Variation of the compressive force of the yamu´s meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage. Different letters represent significant differences.

The incorporation of sorbitol and the storage time caused a significant difference (P<0.05) on the values of the texture of yamú's meat, separately (P<0.05). In turn, its values presented a significant difference (P<0.05) due to the interaction between the incorporation of the sorbitol and the storage temperature. It is noteworthy that the texture values are higher when the meat is treated with sorbitol and stored at -18 °C regardless the time it was stored

because the cryoprotectant acts only at freezing temperature, preventing the formation of intracellular or intercellular ice crystals that may affect the texture. The interaction (P<0.05) of the incorporation of sorbitol with the storage time made possible to observe that the texture of the meat is higher in all cases, in which sorbitol is incorporated, especially when compared with the meat that was stored for 48 h without the incorporation of the cryoprotectant (Figure 2).

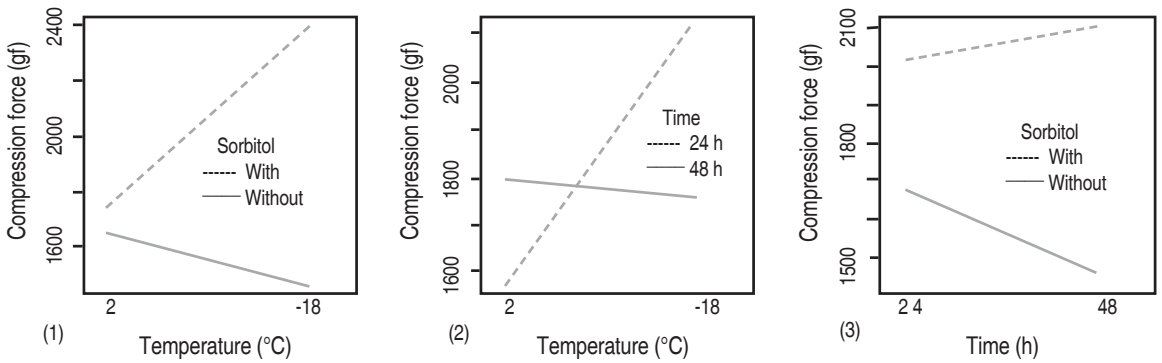

Figure 2. Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) on the variable texture.

The presence of interaction (P<0.05) between temperature and storage time indicates that the freezing temperature maintains a greater texture in the meat during 24 h of storage in comparison with the decrease in texture observed at 48 h of frozen storage, independently of the use or not of sorbitol. The effect of sorbitol is not visible when the meat is stored at a temperature of 2 °C regardless of the storage time, 24h (T2) and 48 h (T6). Besides, significant texture loss was obtained in the treatments that did not use the sorbitol incorporation at 2 °C for storage time of 24 h (T1) and 48 h (T5); the same occurred to the 24 h storage at -18 °C without sorbitol (T3), and with an even higher loss when the meat was stored for 48 h at -18 °C without sorbitol (T7). However, when the incorporation of sorbitol was used at -18 °C for 24 h (T4), the highest compressive strength was obtained, although this is diminished at the 48 h (T8) of storage.

The results in the compression test show higher values in all treatments than those reported for fillets of the same species stored at -8 °C for 61 h (Castañeda *et al*., 2016) and for *Brycon cephalus* stored at -3 °C for 12 h (Suárez Mahecha *et al*., 2006). These results may be influenced by the time elapsed from slaughter to storage, storage time, diet and treatment with sorbitol. However, the data are similar to those reported for *Urophycis chuss* treated with the incorporation of sorbitol and sodium tripolyphosphate (Bigelow and Lee, 2007).

The loss of texture is evident when the meat is subjected to -18 °C without the incorporation of sorbitol due to damages generated by the formation of intracellular and intercellular ice crystals that damage the muscular structure (Tomaniak *et al*., 1998; Leygonie *et al*., 2012); there is a higher damage as storage time elapses. On the other hand, the incorporation of sorbitol in the meat is effective when it is stored at a temperature of -18 °C, thus obtaining values of compressive strength above other treatments, with similar results to those obtained in *Onchorynchus mykiss* fillets, immersed in an 8% solution of sorbitol and sucrose and stored at -20 °C (Jittinandana *et al*., 2003).

Water holding capacity and pH

The analysis of variance performed for the WHC parameters obtained shows that there was a significant difference between treatments (P<0.05). The Tukey test indicates that the treatment subjected to 24 h at 2 °C without the incorporation of sorbitol (T1) obtained the highest WHC (70.4%), while the treatments T3 (-18 °C for 24 h) , T5 (2 °C for 48 h), and T7 (-18 °C for 48 h), that also did not include the incorporation of sorbitol,

presented statistical similarity among them and low WHC with values of 63.05, 65.7 and 62.01%, respectively. Being the lowest value, the one obtained for T7. The treatments that used the sorbitol incorporation T2 (2 °C for 24 h), T4 (-18 °C for 24 h), T6 (2 °C for 48 h), and T8 (-18 °C for 48 h) had WHC values of 66.30, 66.47, 63.46, and 66.92, respectively; these treatments were statistically similar except for T6 (Figure 3).

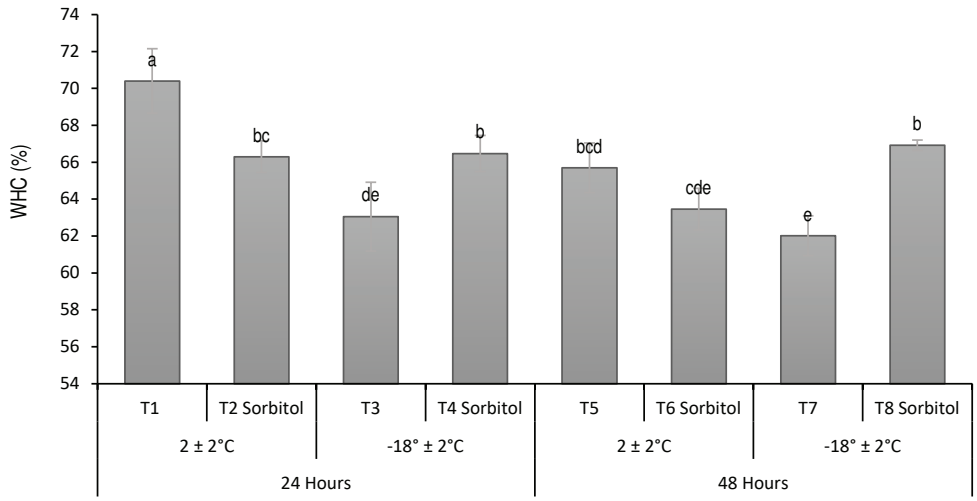

Figure 3. Variation of WHC of yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different storage times and temperatures. Different letters represent significant differences.

The interaction of the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) affected the variable WHC (Figure 4). These values were influenced by temperature and storage time (P<0.05); the interaction between the incorporation of sorbitol with storage temperature also presented a significant effect (P<0.05) because the WHC presented the lowest value when the meat was stored at -18 °C without the incorporation of the cryoprotectant, while this characteristic remained when the meat was treated with sorbitol. The interaction between temperature and storage time (P<0.05) showed that, independently of the storage temperature, WHC decreases after 48 h for all treatments, especially for those who were not treated with sorbitol.

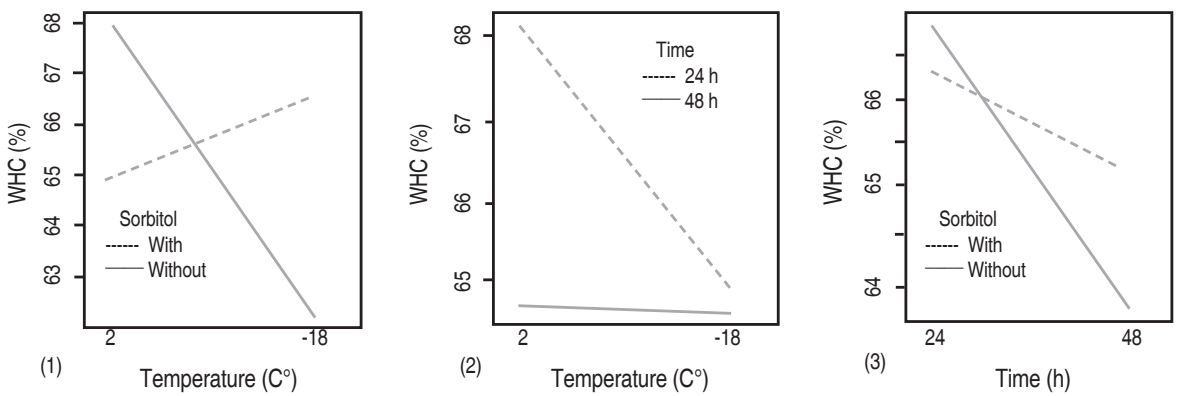

Figure 4. Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of meat of yamú (*Brycon amazonicus*) on the variable water holding capacity.

WHC reported a significant reduction in all treatments, decreasing even more in those that did not have the inclusion of sorbitol. This reduction is explained by the injection of 5% (w/w) of the sorbitol solution that increased the moisture of the treated fillets. This decrease in WHC is due to the loss of functionality of cell membranes, which suffer irreversible damage during freezing, losing their rehydration capacity due to loss of lipid components (Seidel, 2006), and denaturation of myofibrillar proteins such as actin and myosin (Andersen and Jørgensen, 2004; Lund and Baron, 2010). The results of water retention capacity are related to the results obtained in the compression test, where the treatments that maintained the values of WHC (Figure 3) and obtained the highest values of compressive strength (Figure 1) were the same subjected to -18 °C under the incorporation of sorbitol after 24 and 48 h of storage.

The pH variation of yamú's meat was subjected to the incorporation or not of sorbitol at different storage times and temperatures (Figure 5). The Tukey test showed that the treatment subjected to 2 °C for 24 h with the incorporation of sorbitol (T2) obtained the highest pH (6.17), followed by the treatment subjected to 2 °C for 48 h with the incorporation of sorbitol (T6) with a pH value of 6.08. The treatment subjected to -18 °C for 48 h without the incorporation of sorbitol (T6) was the one that obtained the lowest pH (5.93), which showed statistical similarity with the treatment without sorbitol incorporation at -18 °C for 24 h (T3), presenting a pH of 5.95.

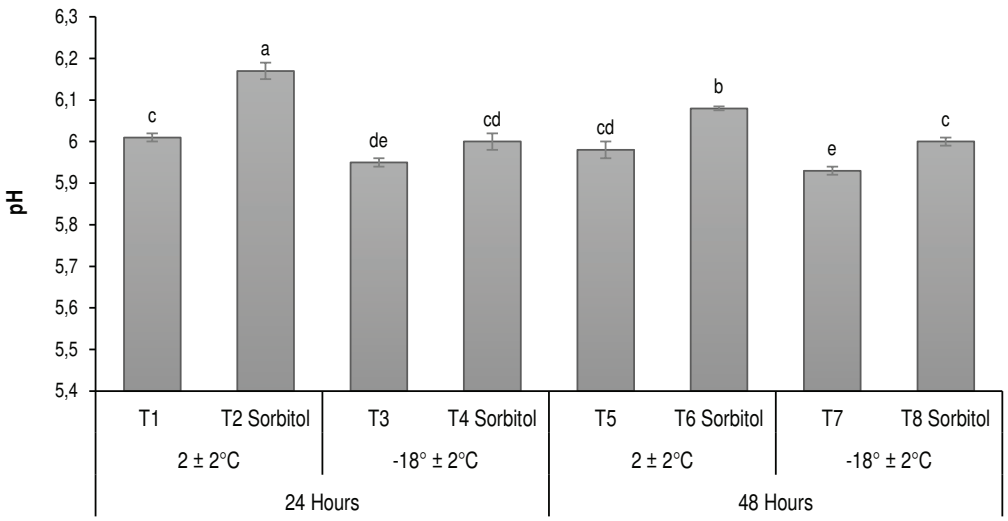

Figure 5. Variation of the pH of the yamú's meat (*Brycon amazonicus*) subjected to the incorporation or not of sorbitol at different times and temperatures of storage. Different letters represent significant differences.

Low pH was evidenced for the treatments without the incorporation of sorbitol at 2 °C and -18 °C for 24 h of storage (6.01 and 5.95 for T1 and T3 respectively) which decreases after 48 h of storage (5.98 and 5.93 for T5 and T7 respectively). The pH for the treatments stored at -18 °C for 24 and 48 h with the incorporation of sorbitol (T4 and T8) was 6.00 (Figure 6), showing a statistical similarity between them.

The pH is higher when sorbitol is added to the yamú meat, regardless of the storage time. However, in the absence of sorbitol, storage at -18 °C causes a reduction in pH, especially when this is prolonged by 48 h. The decrease in pH during storage indicates the transformation of muscle glycogen into lactic acid during the post-mortem stage (Einen *et al*. 2002). Similarly, a low pH is directly related to the loss of texture (Ang and Haard, 1985; Kiessling *et al*., 2004) due to the weakening of connective tissue and protein denaturation (Lavéty *et al*., 1988). The pH remains stable during storage for 48 h when sorbitol is incorporated in the meat subjected to -18 °C.

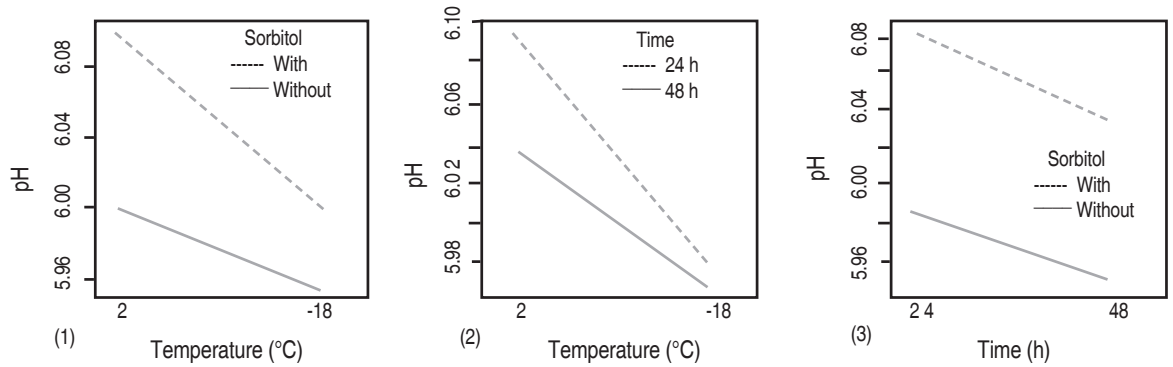

Figure 6. Effect of the interaction between the incorporation or not of sorbitol at different storage times and temperatures of yamú's meat (*Brycon amazonicus*) on the variable pH.

Scanning electron microscopy

The treatments stored for 24 h at 2 °C with or without the incorporation of sorbitol (T1 and T2) showed the myofibrillar structure in its integral state without the separation of the myotomes, while a slight separation of them is observed in the treatments stored for 24 h at -18 °C (T3 and T4). Besides, the changes in the organization of muscle fibers were evidenced in treatments stored at 2 °C for 48 h (T5 and T6). For the treatment stored at -18 °C for 48 h (T7) showed a greater separation of myotomes and myofibrils than the treatment that had the incorporation with sorbitol at the same temperature and storage time conditions (T8), where the separation of the myofibrils was not so evident (Figure 7).

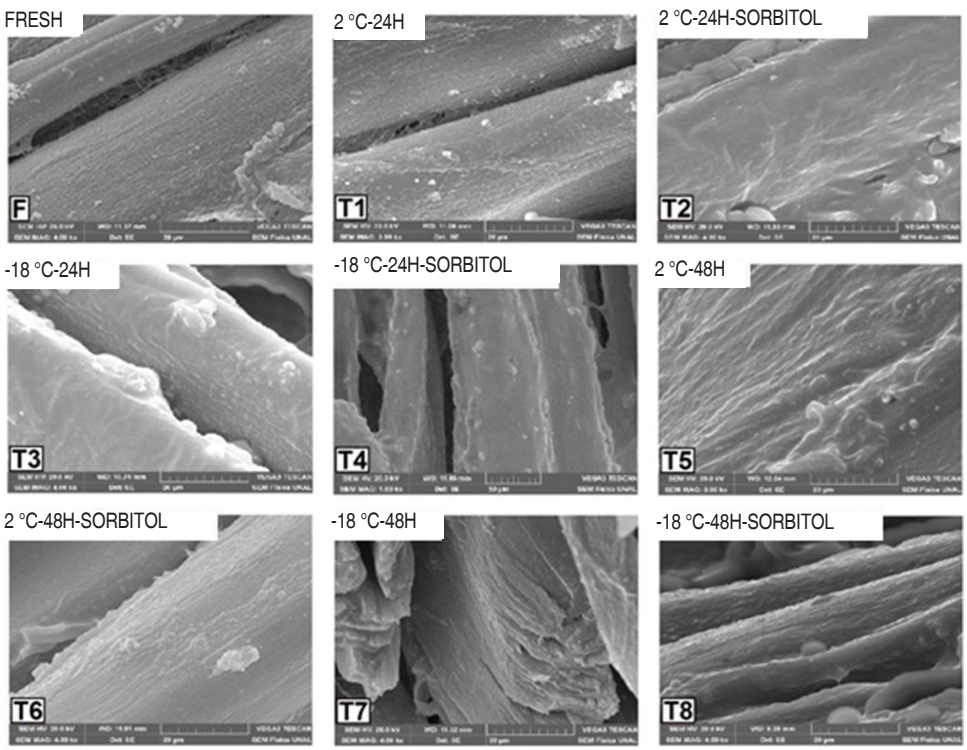

Figure 7. Scanning electron microscopy (SEM) images of yamú meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage times and temperatures.

Optical microscopy

The cross sections of the muscle fibers show slight separation of the endomysium in treatments stored for 24 or 48 h at 2 °C not treated with sorbitol (T1 and T5), whereas treatments with sorbitol incorporation under the same conditions did not present this separation. The structure of the endomysium was affected in larger areas in treatments subjected to -18 °C and not treated with sorbitol regardless of the storage time (T3 and T7) in addition to the presence of interfibrillar separations. Treatments subjected to -18 °C with sorbitol incorporation showed a lower separation of endomysium and interfibrillar spaces (T4), although slightly higher when storage was prolonged for 48 h (T8) (Figure 8).

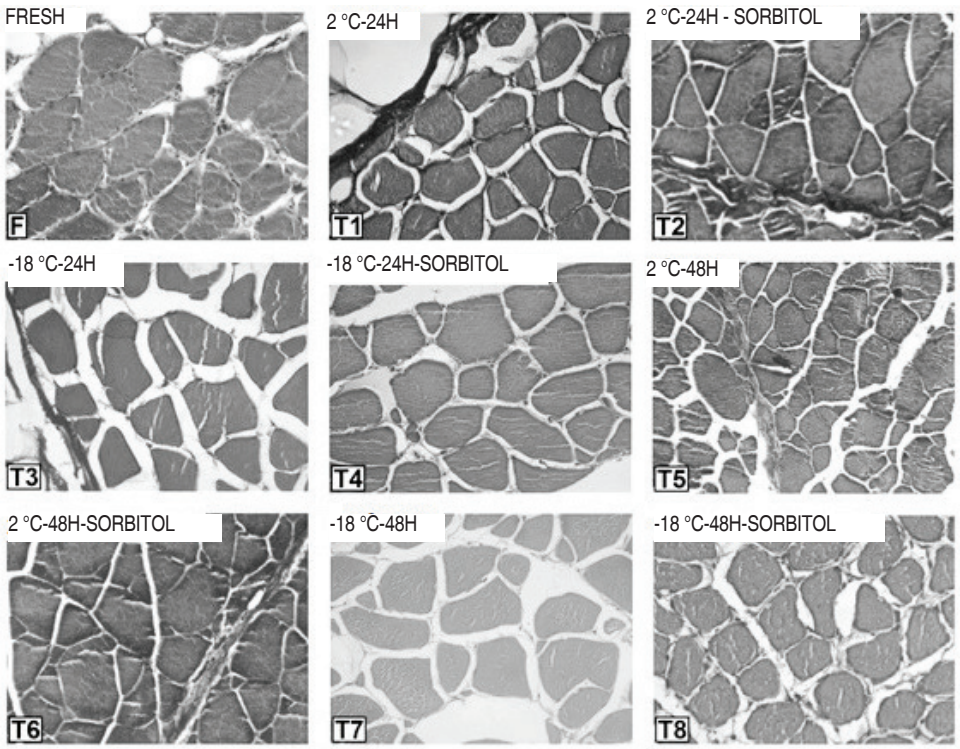

Figure 8. Optical microscopy analysis of yamú meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage imes and temperatures.

The results obtained by optical microscopy and scanning electron microscopy showed the degradation of the connective tissue due to storage at low temperatures; especially at -18 °C, where the loss of interfibrillar tissue, spaces in the myocomata and the separation between myotomes are evident. Those changes in the microstructural tissue are associated with the detachment of the endomysium already reported for other species (Shigemura *et al.,* 2003) and are independent of the storage time (Figure 4 and 5) . Similar results are reported for fillets obtained from the same species and stored at a temperature of -8 °C (Castañeda *et al*., 2016). These changes in muscle structure indicate the degradation of collagen due to storage at low temperatures, which has been reported for several species (Ando *et al*., 1991; Sato, *et al*., 1991; Sato *et al*., 1997; Shigemura *et al*., 2003; Larsson *et al*., 2014). The formation of ice crystals during freezing damages the ultrastructure of the muscle of fish, which causes alterations in the biochemical reactions that occur at the cellular level and influence the physical quality parameters of the meat (Leygonie *et al*., 2012). However, the muscle structure was maintained in a better condition when the meat was treated under the same storage conditions and with the incorporation of sorbitol, finding less separation between myotomes and greater integrity of the interfibrillar tissue.

Electrophoretic mobility profiles by SDS-PAGE

Up to 14 bands were detected in polyacrylamide gel electrophoresis (Figure 9). The bands with the highest molecular weight (243.54 and 220.93 kDa) were found in all treatments, with a higher intensity in treatments stored for 48 h (T5, T6, T7, and T8). In addition, bands with lower molecular weight (156.08 kDa, 104.39 kDa, 99.43 kDa, 79.09 kDa, 76.72 kDa, 71.31 kDa, 67.51 kDa, 60.93 kDa, and 57.68 kDa) were found in all treatments, especially in those subjected to -18 °C (T3 and T4) and with a slightly higher intensity in treatments subjected to -18 °C for 48 h without sorbitol incorporation (T7 and T8). Bands with a molecular weight of 45.76 and 43.96 kDa with low intensity were presented for treatments stored at 2 °C for 24 h (T1 and T2). The same bands were slightly more intense for the treatments stored at -18 °C for 24 h (T3 and T4) and a higher intensity for the treatments stored at 2 and -18 °C and with (T6 and T8) or without (T7).

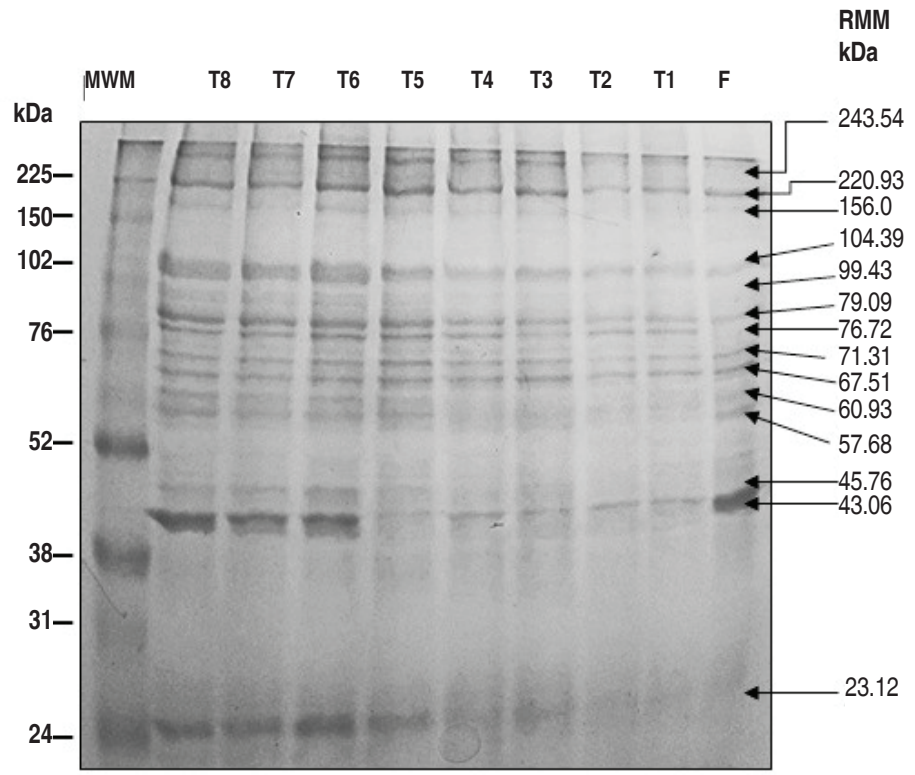

Figure 9. SDS-PAGE gel electrophoresis of 7% acrylamide of protein extracts of yamú's meat (*Brycon amazonicus*), subjected to the incorporation or not of sorbitol at different storage times and temperatures. MWM line: Molecular Weight Marker; RMM: Relative Molecular Mass.

Myofibrillar proteins include contractile proteins such as myosin and actin, regulatory proteins such as troponin, and other minor proteins (Harnedy and Fitzgerald, 2012). Other authors have reported the molecular weight of some proteins present in fish meat, such as long chain myosin 200 kDa (Liu *et al*. 2013), α-actin 105 kDa, troponin 78 kDa (Ladrat *et al.* 2003) and actin 45 kDa (Cao *et al*. 2006). Bands with similar molecular weights can be observed in all treatments with a greater intensity in treatments stored for 48 h (T5, T6, T7, and T8) where the bands with similar molecular weights to heavy chain myosin (220.93 kDa), α-actin (104.39 kDa), troponin (79.09 kDa). and actin (45.76 and 43.06 kDa) were maintained. On the other hand, bands with higher (243.54 kDa) and smaller molecular weight (156.08, 99.43, 76.72, and 23.12 kDa) were also found and are not present in the line corresponding to the analyzed protein profile of the fresh meat (F), and they have greater intensity at 2 °C and -18 °C for 48 h of storage with the addition or not of sorbitol (T5, T6, and T7). It can be inferred that these bands are protein aggregates or partially degraded myofibrillar

protein fragments, which are largely responsible for the functional properties of meat during refrigerated or frozen storage (Mackie, 1993). The fragmentation or aggregation of the myofibrillar proteins generates losses of their functionality and solubility generating softening (Medina and Pazos, 2010; Nikoo *et al*. 2016; Leygonie *et al*. 2012), being lower in the treatment subjected to -18 °C for 24 h with the incorporation of sorbitol (T4).

SUMMARY

The yamú's meat suffered a partial degradation of its myofibrillar proteins such as myosin, α-actin and actin, especially when it was not treated with sorbitol at freezing temperature (-18 °C), besides there were changes in the connective tissue, related to the loss of texture. The physicochemical properties evaluated in yamú's meat were affected mainly by the interaction between temperature and storage time, while the incorporation of sorbitol and the storage temperature had a significant effect on the texture of yamú's meat. The water holding capacity was significantly affected by the time and temperature of storage, and the pH of the meat was significantly affected by all the evaluated factors.

CONCLUSIONS

The main damages caused by the freezing of the muscular structure of the yamú's meat are presented by the loss of the connective tissue and denaturation of the myofibrillar proteins, which generates the loss of functional characteristics such as texture and water holding capacity. The use of sorbitol at -18 °C minimized the negative effects of freezing on the characteristics of the yamú's muscle, maintaining the muscular microstructure in a better condition, generating a cryoprotective effect compared to untreated meat.

REFERENCES

Alvarez MD, Fernández C and Canet W. 2010. Oscillatory rheological properties of fresh and frozen/thawed mashed potatoes as modified by different cryoprotectants. Food and Bioprocess Technology 3(1): 55-70. doi: 10.1007/s11947-007-0051-9

Andersen CM and Jørgensen BM. 2004. On the relation between water pools and water holding capacity in cod muscle. Journal of Aquatic Food Product Technology 13(1): 13–23. doi: 10.1300/J030v13n01_03

Ando M, Toyohara H, Shimizu Y and Sacaguchi M. 1991. Post-mortem tenderization of fish muscle proceeds independently of resolution of rigor mortis. Nippon Suisan Gakkaishi 57(6): 1165–1169. doi: 10.2331/suisan.57.1165

Ang JF and Haard NF. 1985. Chemical composition and postmortem changes in soft textured muscle from intensely feeding Atlantic cod (Gadius morhua, l). Journal of Food Biochemistry 9(1): 49-64. doi: 10.1111/j.1745-4514.1985.tb00338.x

Arias JA. 2006. Estado actual del conocimiento sobre el yamú, *Brycon amazonicus*. Revista Colombiana de Ciencias Pecuarias 19(2): 125–33.

Benjakul S and Visessanguan W. 2010. Chapter 9: Impacts of freezing and frozen storage on quality changes of seafood. pp. 283–306. In: Sakamon Devahastin (ed.). Physicochemical aspects of food engineering and processing. First Edition. CRC Press, Florida. 382 p.

Bigelow W and Lee C. 2007. Evaluation of Various Infused Cryoprotective Ingredients for Their Freeze–Thaw Stabilizing and Texture Improving Properties in Frozen Red Hake Muscle. Journal of Food Science 72(1): C056–C064. doi: 10.1111/j.1750-3841.2006.00216.x

Cao MJ, Jiang XJ, Zhong HC, Zhang ZJ and Su WJ. 2006. Degradation of myofibrillar proteins by a myofibril-bound serine proteinase in the skeletal muscle of crucian carp (*Carasius auratus*). Food Chemistry 94(1): 7-13. doi: 10.1016/j.foodchem.2004.09.016

Castañeda D, Lozano J and Suárez H. 2016. Microstructural changes and the effect on myofibril proteins in yamú (*Brycon amazonicus*) fish meat during cold storage. Agronomía Colombiana 34(3): 403-414. doi: 10.15446/agron.colomb.v34n3.61316

Einen O, Guerin T, Fjæra S and Skjervold P. 2002. Freezing of pre-rigor fillets of Atlantic salmon. Aquaculture 212(1-4): 129-140. doi: 10.1016/S0044-8486(01)00874-2

Goeller LM, Amato PM, Farkas BE, Green DP, Lanier TC and Kong CS. 2004. Optimization of incorporation of low-molecular-weight cryoprotectants into intact fish muscle. Journal of Food Science 69(4): 164-171. doi: 10.1111/j.1365-2621.2004.tb06342.x

Gonçalves AA, Nielsen J and Jessen F. 2012. Quality of frozen fish. pp. 479-509. In: Nollet LML, Boylston T, Chen F, Coggins P, Hydling G, McKee LH and Kerth C (eds.). Handbook of meat, poultry and seafood quality. Second Edition. John Wiley & Sons Inc., Ames. 576 p.

Harnedy PA and Fitzgerald RJ. 2012. Bioactive peptides from marine processing waste and shellfish: A review. Journal of Functional Foods 4: 6–24. doi: 10.1016/j.jff.2011.09.001

Hunt A and Park JW. 2014. Comparative study of sodium bicarbonate on gelling properties of Alaska pollock surimi prepared at different freezing rates. Journal of Food Quality 37(5): 349–360. doi: 10.1111/jfq.12099

Jacobsen C, Nielsen HH, Jørgensen B and Nielsen J. 2010. Chemical processes responsible for quality deterioration in fish. pp. 439-465. In Skibsted LH, Risbo J and Andersen ML (eds.). Chemical deterioration and physical instability of food and beverages. First edition. Cambridge: Woodhead Publishing Limited, New Delhi. 763 p. doi: 10.1533/9781845699260.3.439

Jittinandana S, Kenney P and Slider S. 2003. Cryoprotection affects physiochemical attributes of rainbow trout fillets. Journal of Food Science 68(4): 1208–1214. doi: 10.1111/j.1365-2621.2003.tb09626.x

Kiessling A, Espe M, Ruohonen K and Mørkøre T. 2004. Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. Aquaculture 236(1–4): 645-57. doi: 10.1016/j.aquaculture.2004.02.030.

Kittiphattanabawon P, Benjakul S, Visessanguan W and Shahidi F. 2012. Cryoprotective effect of gelatin hydrolysate from blacktip shark skin on surimi subjected to different freeze-thaw cycles. LWT - Food Science and Technology 47(2): 437–442. doi: 10.1016/j.lwt.2012.02.003

Ladrat C, Verrez-Bagnis V, Noël J and Fleurence J. 2003. In vitro proteolysis of myofibrillar and sarcoplasmic proteins of white muscle of sea bass (*Dicentrarchus labrax* L.): effects of cathepsins B, D and L. Food Chemistry 81(4): 517-25. doi: 10.1016/S0308-8146(02)00481-8

Laemmli U. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680–685.

Larsson T, Koppang EO, Espe M, Terjesen BF, Krasnov A, Moreno Conde HM, Rørvik KA, Thomassen, MS and Mørkøre T. 2014. Fillet quality and health of Atlantic salmon (Salmo salar L.) fed a diet supplemented with glutamate. Aquaculture (426-427): 288-295. doi: 10.1016/j. aquaculture.2014.01.034

Lavéty J, Afolabi OA and Love RM. 1988. The connective tissues of fish. International Journal of Food Science & Technology 23(1): 23-30. doi: 10.1111/j.1365-2621.1988.tb00546.x

Lee J, Park HW, Jenkins R, Yoon WB and Park JW. 2017. Image and chemical analyses of freezing-induced aggregates of fish natural actomyosin as affected by various phosphate compounds. Food Bioscience 19: 57–64. doi: 10.1016/j.fbio.2017.06.007

Leygonie C, Britz TJ and Hoffman LC. 2012. Impact of freezing and thawing on the quality of meat: Review. Meat Science 91(2): 93-98. doi: 10.1016/j.meatsci.2012.01.013

Liu D, Liang L, Xia W, Regenstein JM and Zhou P. 2013. Biochemical and physical changes of grass carp (*Ctenopharyngodon idella*) fillets stored at -3 and 0 °C. Food Chemistry 140(1–2): 105-14. doi: 10.1016/j.foodchem.2013.02.034

Lund MN and Baron CP. 2010. Protein oxidation in foods and food quality. pp. 33–69. In Skibsted LH, Risbo J and Andersen ML (eds.). Chemical deterioration and physical instability of food and beverages. First Edition. Woodhead Publishing Limited, New Delhi. 824 p. doi: 10.1533/9781845699260.1.33

Mackie IM. 1993. The effects of freezing on flesh proteins. Food Reviews International 9(4): 575–610. doi: 10.1080/87559129309540979

Medina I and Pazos M. 2010. Oxidation and protection of fish. pp. 91-120. In Decker E, Elias R and McClements DJ (eds.). Oxidation in foods and beverages and antioxidant applications. First Edition. Cambridge: Woodhead Publishing Limited, New Delhi. 552 p. doi: 10.1533/9780857090331.1.91

Mohan M, Ramachandran D, Sankar T and Anandan R. 2007. Influence of pH on the solubility and conformational characteristics of muscle proteins from mullet (*Mugil cephalus*). Process Biochemistry 42(7): 1056–1062. doi: 10.1016/j.procbio.2007.04.005

Nikoo M and Benjakul S. 2015. Potential application of seafood-derived peptides as bifunctional ingredients, antioxidant–cryoprotectant: A review. Journal of Functional Foods 19(Part A): 753–764. doi: 10.1016/j.jff.2015.10.014

Nikoo M, Benjakul S and Rahmanifarah K. 2016. Hydrolysates from marine sources as cryoprotective substances in seafoods and seafood products. Trends in Food Science & Technology 57(Part A): 40–51. doi: 10.1016/j.tifs.2016.09.001

Ramírez JA, Santos IA, Morales OG, Morrissey MT and Vázquez M. 2000. Application of Microbial Transglutaminase to Improve Mechanical Properties of Surimi from Silver carp. Ciencia y Tecnologia Alimentaria 3(1): 21–28. doi: 10.1080/11358120009487644

Sánchez-Alonso I, Martinez I, Sánchez-Valencia J and Careche M. 2012. Estimation of freezing storage time and quality changes in hake (*Merluccius merluccius*, L.) by low field NMR. Food Chemistry 135(3): 1626–34. doi: 10.1016/j.foodchem.2012.06.038

Sato K, Ohashi C, Ohtsuki K and Kawabata M. 1991. Type V collagen in trout (Salmo gairdneri) muscle and its solubility change during chilled storage of muscle. Journal of Agricultural and Food Chemistry 39(7): 1222–1225. doi: 10.1021/jf00007a005

Sato K, Ando M, Kubota S, Origasa K, Kawase H, Toyohara H, Sakaguchi M, Nakagawa T, Makinodan Y, Ohtsuki K and Kawabata M. 1997. Involvement of type V collagen in softening of fish muscle during short-term chilled storage. Journal of Agricultural and Food Chemistry 45(2):343–348. doi: 10.1021/jf9606619

Seidel GE. 2006. Modifying oocytes and embryos to improve their cryopreservation. Theriogenology 65(1): 228–235. doi: 10.1016/j.theriogenology.2005.09.025

Shigemura Y, Ando M, Tsukamasa Y, Makinodan Y and Kawai T. 2003. Correlation of type V collagen content with postmortem softening of fish meat during chilled storage. Fisheries science 69(4): 842–848. doi: 10.1046/j.1444-2906.2003.00696.x

Suárez Mahecha H, Pardo Carrasco SC, Beirão LH, de Francisco A and Okada Nagaky LL. 2006. Efecto de la súper refrigeración sobre la textura de la carne de matrinxã (*Brycon cephalus*). Revista Colombiana de Ciencias Pecuarias 19(2): 221–227.

Tomaniak A, Tyszkiewicz I and Komosa J. 1998. Cryoprotectants for frozen red meats. Meat Science 50(3): 365–371. doi: 10.1016/S0309-1740(98)00043-6

Velasco J, Dobarganes C and Martinez-Ruiz G. 2010. Oxidative rancidity in foods and food quality. pp. 3-32. In: Skibsted LH, Risbo J and Andersen ML (eds.). Chemical deterioration and physical instability of food and beverages. First Edition. Woodhead Publishing Limited, New Delhi. 824 p.

Watabe S. Hwang GC, Nakaya M, Guo XF and Okamoto Y. 1992. Fast Skeletal Myosin Isoforms in Thermally Acclimated Carp. The Journal of Biochemistry 111(1):113-122. doi: 10.1093/oxfordjournals.jbchem.a123706

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Jairo Humberto López. Cryoprotective effect of sorbitol on the muscle microstructure of yamú (*Brycon amazonicus*) during storage at 2 and -18 °C. 72(1): 8763-8774. 2019.

Jaramillo Mesa Helena. Complete genome sequence of a *Passion fruit yellow mosaic virus* (PFYMV) isolate infecting purple passion fruit (*Passiflora edulis* f. *edulis*). 72(1): 8643-8654. 2019.

José Manuel Lozano. Cryoprotective effect of sorbitol on the muscle microstructure of yamú (*Brycon amazonicus*) during storage at 2 and -18 °C. 72(1): 8763-8774. 2019.

Julián Guillermo León. Cryoprotective effect of sorbitol on the muscle microstructure of yamú (*Brycon amazonicus*) during storage at 2 and -18 °C. 72(1): 8763-8774. 2019.

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Ramos Ana. Behavior of bioactive compounds and antioxidant activity of mango (Azucar cultivar) juice during storage at 4 °C. 72(1): 8743-8750. 2019.

Rojano Benjamín Alberto. Behavior of bioactive compounds and antioxidant activity of mango (Azucar cultivar) juice during storage at 4 °C. 72(1): 8743-8750. 2019.

Santos Collier Leonardo. Effect of nutrient cycle influenced by inter-row cover crops on the nutritional status of rustic grapevine. 72(1): 8685-8698. 2019.

Schneider Theodoro. Mixture of glufosinate and atrazine for ryegrass (*Lolium multiflorum* Lam.) control and its effect on seeds' quality. 72(1): 8655-8661. 2019.

Seleguini Alexsander. Effect of nutrient cycle influenced by inter-row cover crops on the nutritional status of rustic grapevine. 72(1): 8685-8698. 2019.

Vargas Leandro. Mixture of glufosinate and atrazine for ryegrass (*Lolium multiflorum* Lam.) control and its effect on seeds' quality. 72(1): 8655-8661. 2019.

Zapata Londoño María Bibiana. Behavior of bioactive compounds and antioxidant activity of mango (Azucar cultivar) juice during storage at 4 °C. 72(1): 8743-8750. 2019.

POLÍTICA EDITORIAL

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La Revista Facultad Nacional de Agronomía (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales e inéditos de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias.

Artículos de reflexión: Documento que presenta resultados de investigación terminada desde una perspectiva analítica, interpretativa o crítica del autor, sobre los temas específicos ya citados, recurriendo a fuentes originales.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

El Comité Editorial, apoyado por un equipo de editores asociados, evaluará el mérito científico del documento y luego lo someterá a evaluación bajo la modalidad doble ciego -es decir que se guarda estricto anonimato en la revisión- por dos árbitros especializados en el tema, preferiblemente uno nacional y otro internacional, quienes entregarán su dictamen en el formato establecido por la Revista. El Comité Editorial se reserva el derecho de aceptar o no las colaboraciones. El dictamen luego del proceso de revisión puede ser: aceptado para publicación sin ninguna o pocas modificaciones; aceptado para publicación con cambios mayores de acuerdo a las observaciones de los evaluadores; reconsiderado para publicación si se modifica sustancialmente, en este caso, será catalogado como material nuevo; rechazado para publicación. Si los artículos son aceptados, estos serán devueltos a los autores para su corrección y remitidos de nuevo al Director de la Revista en los siguientes 30 días calendario.

La impresión de gráficos, figuras o fotografías en color es opcional y tiene un costo adicional por página necesaria de cien mil pesos colombianos ($100.000). La redacción de la Revista se reserva el derecho de realizar modificaciones de forma en el texto del artículo (títulos, resúmenes/abstracts, tablas y figuras); siempre que sea posible, se consultará a los autores sobre los cambios introducidos.

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INSTRUCCIONES A LOS AUTORES

Lineamientos generales

Los artículos deben ser enviados a través del Open Journal System en el Portal de Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se debe remitir el formato "Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales", en el cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión, artículos de reflexión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agrícolas, Producción Animal, Forestales y de Ingeniería Agrícola y de Alimentos. La extensión no debe exceder de 5.200 palabras, las hojas deben ser tamaño carta, escritas a interlineado sencillo, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas cuando se requiera y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Cuando se requiera se debe usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: "ocho tratamientos", "3, 7 y 9 lecturas", "15 kg". Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra "a", y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave mínimo cuatro, distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

Se deben describir en forma clara, concisa y secuencial, los materiales utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Se debe tener la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para $P<0,05$, doble asterisco (**) para $P<0,01$ o triple asterisco (***) para $P<0,001$. Las

investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Literatura citada

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase, artículos en preparación o en prensa, o cualquier otra publicación de circulación limitada. Se debe evitar el exceso de autocitas.

La bibliografía se deberá incluir al final del texto, sólo con las referencias citadas en el mismo. Se debe incluir el número doi asignado a cada artículo consultado. Las citas en el texto deben incluir apellido del autor y año, con coma entre autor y año. Ejemplo: Pérez, 1995; además conservar el siguiente orden de citación:

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- Si hay dos autores se citarán separados por la conjunción y. Ejemplo: Gil y Ortega, 1993.
- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: Gómez, 2000a, 2000b, 2000c.

En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* que significa y otros; en la bibliografía se deben citar todos los autores.

Las comunicaciones personales, se deben citar al pie de la página y no se incluyen en la bibliografía.

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, de debe seguir el orden cronológico creciente, si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de la publicación original.

En cada referencia para todos los autores cite primero el apellido seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Para libros: Autor (es). Año. Título del libro, edición, ciudad de su sede, casa editora y, páginas consultadas (pp. # - #) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

Para capítulos de libros: Autor (es). Año. Título del capítulo, páginas consultadas (pp. # - #). En: Apellidos y nombres de los compiladores o editores (eds.), título del libro, edición, casa editora y ciudad de su sede, páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Para artículos de revistas: Autor (es). Año. Título del artículo, nombre completo de la revista volumen(número): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo. Santa Fé de Bogotá.

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia. Trabajo de grado Ingeniería Agronómica. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Cita de cita. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica 9(2): 227- 232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agrarias, Universidad Nacional de Colombia. Medellín. 46 p.

Suplemento de revista. Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Supl. 2: 195-201.

Para citas de internet: Autor (es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Agricultura Tropical, http://agrotropical.edunet.es. 25 p.; consulta: noviembre 2003.

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The National Faculty of Agronomy Journal (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia - Medellín. It is aimed at teachers, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

The Journal is a four-monthly publication at national and international level. Its aim is to disclose original and unpublished articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

Taking into account Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) criteria, the journal welcomes papers of the following types:

Research papers in science and technology: A document presenting in detail the original results of completed research projects. The structure generally used contains four main parts: Introduction, methodology (materials and methods), results and discussion, and conclusions.

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Critical reflection articles: A document presenting completed research results from an analytical, interpretive or critical author's point of view, on the specific issues already mentioned, using original sources.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

Articles must be submitted in accordance with the guidelines set forth in "Instructions to Authors"; those who violate the rules will not initiate the basic editorial process. Shall be filled the form "Authorization for Release of Works and Economic Rights Assignment", which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

The Editorial Board, supported by a team of associate editors, will evaluate the scientific merit of the paper and will then submit it for evaluation under double-blind method- that is to say, strict anonymity in the review is kept- by two arbitrators specialized in the area, preferably one national and one international, who will give their report on the format provided by the Journal. The Editorial Board reserves the right to accept collaborations. The report, after the review process, can be: accepted for publication with no or few modifications; accepted for publication with major changes according to the comments of the evaluators; reconsidered for publication if it is substantially modified - in this case, it will be deemed as new material; rejected for publication. If articles are accepted, they will be returned to authors for correction and sent again to the Director of the Journal within 30 calendar days.

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INSTRUCTIONS TO AUTHORS

General guidelines

Papers can be sent by email to: rfnagron_med@unal.edu.co, or through the Open Journal System in the Universidad Nacional de Colombia journals web side http://www.revistas.unal.edu.co/, Will be considered only papers written in English. Letter of originality which accepts no simultaneous nomination of the article to other journals or publishers and assigns and the "Autorization for Release of Works and Economic Rights Assignment" should be attached. Publishing forms are: scientific and technological research articles, review articles, reflection articles, and short articles. Articles can be developed by professors and/or researchers at the Universidad Nacional de Colombia, or other related national or international institution, on Agricultural, Forestry, Food and Agricultural Engineering matters. Article extension must not exceed 5,200 words, it must be letter-size sheets, typed double-spaced, 12 point Times New Roman or Verdana font, 3 cm margin at the upper, 2 cm in the lower, 2.5 cm on the left and right side margins. Tables and figures (i.e, graphics, drawings, diagrams, flowcharts, photographs and maps) should be shown on separate sheets and numbered consecutively (Table 1 ... Table n, Figure 1…Figure n, etc.). Texts and tables should be submitted in MS-Word® word processor, original tables and diagrams of frequency (bar charts and pie charts) must be supplied in manuscript file and in its original MS-Excel®; other figures, such as photographs on paper and drawings, can be sent in original or scanned and sent in digital format compression JPG (or JPEG), preferably with a resolution of 600 x 600 dpi (300 dpi at least); original photographs are suggested to be sent as slides. As a general rule, tables and figures are only accepted in black and white. Color figures will be exceptionally accepted when strictly necessary and under discretion of the Editorial Board.

Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: "eight treatments", "3,7 and 9 readings", "15 kg". Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter "a" and hyphen for growing seasons. Example period 2002a2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English, Spanish or Portuguese. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other researches, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Cited Literature

Only bibliographical references cited in the text are listed. Lecture notes, articles in preparation or in press, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

The bibliography should be included at the end of the text, containing only the references cited in it, including the doi number. Citations in the text should include author's surname and year, with comma between author and year. Example: Pérez, 1995. They should also keep the following citation order:

- If more than one date, they are separated by commas: Example: Pérez, 1995, 1998, 2001.

- If there are two authors, they will be separated by the conjunction and. Example: Gil and Ortega, 1993.

If there are several works by an author, published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: Gómez, 2000a, 2000b, 2000c.

For citations with three or more authors, it is necessary to mention in the text the surname of the first and replace the others by the Latin expression *et al.,* which means and others. All authors should be mentioned in the bibliography.

Personal communications should be cited at the bottom of the page and not included in the bibliography.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications by the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

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References should contain all the data allowing to its easy location.

Examples:

For books: Author (s), year. Book title, edition, place of publication, publisher and pages consulted (pp. # - #) or total pages (# p.). Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

For book chapters: Author (s), year. Chapter title, pages consulted (pp. # - #). En: Surnames and names of the editors or publishers (eds.), book title, edition, publisher and place of publication, total pages (# p.). Example: Bernal H. 1996. Chapter 6: Evapotranspiración. pp. 112-125. In: Agrios G. (ed.). Fitopatología. Second Edition. Editorial Limusa, México D.F. 400 p.

For journals: Author (s), year. Article title, journal full name volume(number): page-page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. Geotechnical engineering and environmental protection. p. 65-94. In: Memorias IX Colombian Congress of Soil Science. Colombian Society of Soil Science. Santa Fé de Bogotá.

Theses and dissertations: Gómez C. 2004. Autoecología de mortiño (*Vaccinium meridionale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 78 p.

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Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Tropical Agriculture, http://agrotropical. edunet.es. 25 p.; accessed: November 2003.

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

A Revista Facultad Nacional de Agronomía é uma publicação da Facultad de Ciencias Agrarias da Universidad Nacional de Colombia – Sede Medellín. Orienta-se a professores, pesquisadores, estudantes e a todos os profissionais que criam conhecimento e articulam a ciência e a tecnologia para fazer o campo mais produtivo no âmbito empresarial e da economia camponesa.

A periodicidade da Revista é cuatrimestral, com circulação nacional e internacional e seu objetivo é divulgar artigos originais e inéditos de caráter científico que respondam perguntas específicas e forneçam suporte e provas a uma hipótese, em aspectos relacionados com das Ciências Agronômicas, Zootecnia, Ciências Florestais e Engenharia Agrícola e de Alimentos e disciplinas afins que contribuam à solução dos limitantes do agro no trópico.

Levando em conta os critérios considerados por Colciencias, a revista considera documentos das seguintes tipologias:

Artigos de pesquisa científica e tecnológica: Documentos que apresentam, de forma detalhada, os resultados originais de projetos de pesquisa concluídos. A estrutura utilizada contém, geralmente, quatro partes fundamentais: introdução, metodologia (materiais e métodos), resultados e discussão, e conclusões.

Artigos de revisão: Documentos produto de uma pesquisa concluída onde são analisados, sistematizados e integrados os resultados de pesquisas publicadas ou não publicadas, sobre um campo em ciência e tecnologia, a fim de dar conta dos avanços e tendências de desenvolvimento. Caracteriza-se por apresentar uma cuidadosa revisão bibliográfica de pelo menos 50 referências.

Artigos de reflexão: Documentos que apresentam resultados de pesquisa concluída com uma perspectiva analítica, interpretativa ou crítica do autor, sobre os temas específicos antes mencionados, recorrendo a fontes originais.

Artigos curtos: Documentos breves que apresentam resultados originais preliminares ou parciais de uma pesquisa cientifica ou tecnológica, e que geralmente precisam de uma rápida difusão. Para todos os casos o 60% das citações deve provir de artigos publicados nos últimos dez anos.

Os artigos devem ser apresentados de acordo com os parâmetros estabelecidos nas "Instruções para os Autores", aqueles que não seguirem as normas básicas não serão considerados para publicação. Deve preencher o formulário "Autorização para Publicação de Obras e Sessão de direitos" a qual será fornecida pela Revista. O formulário é explícito enquanto que todos os autores estão informados do envio do artigo para a Revista, além de estar de acordo com ele. Também o formulário indica que não se apresentam conflitos de interesse entre eles e expressam que o conteúdo do manuscrito não tem sido nem será enviado para a sua publicação em outra revista.

O Comitê Editorial, junto a uma equipe de editores asociados, avaliará o mérito científico do documento e o submeterá a avaliação na modalidade duplo-cego–isto é, tem- se estrito anonimato da relatoría- por dois árbitros especializados no assunto, um deles nacional e outro internacional, os quais entregarão seu ditame no formato estabelecido pela Revista.

O Comitê Editorial reserva-se o direito de aceitar ou não as colaborações. O ditame após o processo de revisão pode ser: aceito para publicação com nenhuma ou poucas modificações; aceito para publicação com modificações maiores segundo as observações dos avaliadores; reconsiderado para publicação se modificado substancialmente –neste caso será catalogado como material novo-; recusado para publicação. Caso o artigo for aceito, este será devolvido aos autores para correção e remitido novamente ao Diretor da Revista nos seguintes 30 dias calendário.

A impressão de gráficos, figuras ou fotografías em cor é opcional e tem custo adicional por página precisava de cem mil pesos colombianos (100 mil pesos). A redação da Revista reserva-se o direito de realizar modificações de forma no texto do artigo (títulos, resumos/abstracts, tabelas e figuras), embora, sempre que seja possivel, os autores serão consultados a respeito das mudanças introducidas.

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INSTRUÇÕES PARA OS AUTORES

Parâmetros gerais

Os artigos podem ser enviados ao endereço eletrônico: rfnagron_med@unal.edu.co ou também ingressando no site das Revistas da Universidad Nacional de Colombia usando o programa Open Journal System http://www.revistas.unal.edu.co/. Serão considerados apenas os artigos escritos em Inglés. Junto com o trabalho deverá encaminhar o formulário "Autorização para Publicação de Obras e Sessão de direitos" no qual se aceita a não postulação simultânea do artigo a outras revistas ou órgãos editoriais e cedem-se à Revista os direitos de difusão. As formas de publicação são: artigos de pesquisa científica e tecnológica, artigos de revisão, artigos de reflexão e artigos curtos. Os artigos podem ser elaborados por professores e/ou pesquisadores da Universidad Nacional de Colombia, ou qualquer outra instituição afim, nacional ou internacional, nos temas agropecuários, florestais, e de engenharia agrícola e de alimentos. A extensão não deve superar as 5.200 palavras, as folhas devem ser tamanho carta, escritas a duplo espaço, letra ou fonte Times New Roman ou Verdana, tamanho 12 pontos, margens de 3 cm na parte superior, 2 cm na inferior e 2,5 cm nas margens laterais direita e esquerda. As tabelas e figuras (isto é, gráficos, desenhos, esquemas, diagramas de fluxo, fotos e mapas) devem aparecer em folhas independentes e com numeração consecutiva (Tabela 1... Tabela n; Figura 1... Figura n. etc.). Os textos e tabelas devem ser apresentados no processador de palavras MS-Word®; as tabelas e diagramas de freqüência (gráficos de barras e de pizzas) originais devem aparecer tanto no arquivo do manuscrito quanto no original de MS-Excel®; outras figuras, como fotos sobre papel e desenhos, podem ser enviadas em original ou digitalizadas, e remitidas no formato digital de compressão JPG (ou JPEG) preferivelmente com uma resolução de 600 x 600 dpi (mínimo 300 dpi); é desejável que as fotos originais sejam enviadas como slides. Como norma geral, só serão aceitas tabelas e figuras em preto e branco; imagens coloridas serão incluídas só em caso estritamente necessário e a juízo do Comitê Editorial.

Unidades, abreviaturas e estilo

Deve utilizar-se o Sistema Internacional de Unidades (SIU), e aquelas unidades específicas de maior uso por parte da comunidade científica. Quando seja necesario debe-se usar a forma exponencial Exemplo: kg ha^{-1}. O significado das abreviaturas deve ser citado por extenso quando mencionadas por primeira vez no manuscrito. O estilo da escrita deve ser absolutamente impessoal, em tempo gramatical pretérito na introdução, procedimentos e resultados, e presente na discussão, evitando a conjugação de verbos em primeira ou terceira pessoa do singular ou do plural.

Os números de um a nove devem-se escrever em palavras, exceto quando refletem ou indicam unidades de medida ou se colocam vários números consecutivamente Exemplo: "oito tratamentos", "3, 7 y 9 leituras", "15 kg". Deve-se utilizar o zero antes do ponto decimal. Para separar intervalos de um o mais anos, deve-se usar a letra "a", e hífen para períodos de crescimento (safras). Exemplo. Período 2002 a 2005, safras 1999-2000, 2000-2001.

Título e autores

O título do artigo não deve incluir abreviaturas e é obrigatória sua tradução ao inglês. Sempre que possível, o título não deve superar as 15 palavras e deve refletir com precisão o conteúdo do documento. Em caso de conter nomes científicos de espécies vegetais ou animais, estes devem ir em itálica minúscula, com maiúscula somente a primeira letra do gênero e do classificador. Embaixo do título em inglês escreve-se o nome(s) e sobrenome(s) dos autores, sem seus títulos acadêmicos, nem cargos laborais, numa linha horizontal e conforme a sua contribuição à pesquisa e/ou preparação do artigo.

Na parte inferior da primeira página, como nota ao rodapé, escreve-se o cargo laboral dos autores, o nome e a cidade onde se localiza a entidade para a qual trabalham ou do patrocinador para a realização do trabalho e o correspondente endereço eletrônico. Adicionalmente, deve anexar-se um resumo do currículo dos autores, onde se mencionem os artigos publicados em outras revistas.

Resumo, abstract e palavras-chave

O resumo não deve superar as 250 palavras escritas num único parágrafo. Deve ser redigido em espanhol, inglês ou português. Deve conter em forma breve justificação, objetivos, métodos utilizados, resultados obtidos mais relevantes e conclusões. É obrigatório acompanhar o resumo com um máximo de seis palavras-chave, traduzidas ao inglês (key words), diferentes às utilizadas no título. Aceitam-se como palavras-chave não somente palavras simples, mas também termos compostos por até três palavras. Estas devem ir escritas em minúscula e separadas por vírgulas.

Introdução

O título não é obrigatório. Define o problema e informa sobre o estado da arte a respeito do tema principal do artigo, além disso, indica as razões que justificam a pesquisa e propõe os objetivos da mesma. É obrigatório acompanhar os nomes vulgares com o nome(s) científico(s) e a abreviatura(s) do classificador na primeira menção dentro do texto. Não mencionar marcas de produtos, mas nomes genéricos ou químicos.

Materiais e métodos

Aqui devem ser descritos em forma clara, concisa e seqüencial, os materiais (vegetais, animais, implementos agrícolas ou de laboratório) utilizados no desenvolvimento do trabalho, assim mesmo mencionam-se os aspectos relacionados com a localização, preparação e execução dos experimentos. Devem indicar-se o desenho escolhido, as variáveis registradas, as transformações feitas aos dados, os modelos estatísticos usados e o nível de significância empregado. Evitar detalhar procedimentos previamente publicados.

Resultados

São a parte central do artigo, devem ir respaldados por métodos e análises estatísticas apropriadas. Devem apresentar-se de maneira lógica, objetiva e seqüencial mediante textos, tabelas e figuras; estes dois últimos apoios devem ser de fácil leitura, interpretáveis de forma autônoma e ir citados sempre no texto. As tabelas devem conter poucas colunas e linhas. É preciso incluir o nível de significância estatística representado por letras minúsculas do começo do alfabeto (a, b, c, d,...), asterisco simples (*) para $P< 0,05$, duplo asterisco (**) para $P< 0,01$ ou três asteriscos (***) para $P<0,001$. As pesquisas que não obedecem um

desenho estatístico devem mostrar a informação de forma descritiva. Deve-se utilizar subíndice para modificações, os superíndices devem ser utilizados para potencias ou notas ao rodapé em tabelas e figuras.

Discussão

Refere-se à análise e interpretação objetiva dos resultados, confrontando-os com os resultados obtidos em outras pesquisas, ou com os fatos ou teorias conhecidas sobre o tema. Explica os resultados, particularmente quando diferem da hipótese proposta. Destaca a aplicação prática ou teórica dos resultados obtidos e as limitações encontradas. Ressalta a contribuição a uma determinada área do conhecimento e o aporte à solução do problema que justifica a pesquisa. Finalmente, proporciona elementos que permitem propor recomendações ou lançar novas hipóteses. Não devem ser feitas afirmações que vão além do que os resultados podem apoiar.

Conclusões

São as afirmações originadas a partir dos resultados obtidos, devem ser coerentes com os objetivos propostos e a metodologia empregada; adicionalmente, expressar a contribuição ao conhecimento na área temática estudada e propor diretrizes para novas pesquisas.

Agradecimentos

Caso for necessário, incluir-se-ão os agradecimentos ou reconhecimentos a pessoas, instituições, fundos ou bolsas de pesquisa que fizeram contribuições importantes na concepção, financiamento ou realização da pesquisa.

Literatura citada

Devem aparecer somente as referências bibliográficas mencionadas no texto. Não se aceitam notas de aula, artigos em construção ou no prelo, ou qualquer outra publicação de circulação limitada. Evitar o excesso de auto-citas.

A bibliografia deverá aparecer no final do texto, só com as referências citadas no mesmo. As citações no texto devem incluir sobrenomes do autor e ano, com vírgula entre autor e ano. Exemplo: Pérez, 1995; além de conservar a seguinte ordem de citação:

-Se houver mais de uma data, estas se separam com vírgula. Exemplo: Pérez, 1995, 1998, 2001.

-Se houver dois autores, estes se citam separados pela conjunção e. Exemplo: Gil e Ortega, 1993.

-Se houver vários trabalhos de um autor publicados no mesmo ano, estes se citam com uma letra em seqüência alfabética dos títulos, do lado do ano. Exemplo: Gómez, 2000a, 2000b, 2000c.

-Em caso de citações com três ou mais autores, é preciso mencionar no texto os sobrenomes do primeiro e substituir os outros pela expressão latina abreviada *et al.* que significa y outros; já na bibliografia devem aparecer citados todos os autores.

-As comunicações pessoais devem aparecer citadas no rodapé de página e não se incluem na bibliografia.

-As referências bibliográficas devem ir ordenadas alfabeticamente pelo sobrenome do primeiro autor, sem numeração e sem espaçamento na primeira linha. Para citar várias publicações do mesmo autor segue-se a ordem cronológica crescente, e no caso forem do mesmo ano seguirá a ordem alfabética dos títulos.

As referências deverão conter todos os dados que permitam sua fácil localização.

Exemplos:

Para livros: Autor(es), ano. Título do livro, edição, cidade de sua sede, casa editora e, páginas consultadas (pp. # - #) ou páginas totais (# p.). Exemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

Para capítulos de livros: Autor(es), ano. Título do capítulo, páginas consultadas (pp. # - #). Em: Sobrenomes e nomes dos compiladores ou editores (eds.), título do livro, edição, casa editora e cidade de sua sede, páginas totais (# p.). Exemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. Em: Agrios G. (ed.). Fitopatología. Segunda edição. Editorial Limusa, México D.F. 400 p.

Para revistas: Autor(es), ano. Título do artigo, nome completo da revista (volume)número: página-página. Exemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. http://dx.doi.org/10.1093/treephys/24.4.38

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Teses, trabalhos de formatura. Gómez C. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia, Trabalho de formatura. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Citação de citação. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica. 9(2): 227-232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia. Medellín. 46 p.

Suplemento de revista. Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52, Supl. 2: 195-201.

Para citas de internet: Autor(es), ano. Título do artigo. Em: Nome(s) da publicação eletrônica, da página web, portal ou página e sua URL, páginas consultadas (pp.#) ou páginas totais (# p.); data de consulta. Exemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. Em: Agricultura Tropical, http://agrotropical. edunet.es. 25 p.; consulta: novembro 2003.

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

b) Redacción o revisión del contenido intelectual.

c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

———. «Ethics. Conducting research». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#conducting-research.

———. «Ethics. Writing an article». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#writing-an-article.

———. «Fragmentación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updatedURL.pdf.

———. «Fraude en investigación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

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[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

References

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

———. «Conflicto de intereses. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

———. «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

———. «Ethics. Conducting research». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#conducting-research.

———. «Ethics. Writing an article». Accedido 8 de agosto de 2014. http://www.elsevier.com/journal-authors/ethics#writing-an-article.

———. «Fragmentación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updatedURL.pdf.

———. «Fraude en investigación. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

———. «Plagio. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

---

[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

[3] William Black, Rodolfo Russo, y David Turton, «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes», *Physics Letters B* 694, n.º 3 (noviembre de 2010): 246-51.

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

[7] Elsevier, «Plagio. Ethics in research & publication», accedido de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.