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EDITORIAL

Relaciones en tiempos de pandemia: COVID-19 y bienestar animal, ambiental y humano

Toda acción conlleva a una reacción y trae consecuencias. Bajo esta premisa, se hace importante la reflexión en tiempos de pandemia. Un pequeño virus ha logrado lo que ningún gobierno, ninguna iniciativa habían logrado antes: aquietarnos en nuestras casas, ralentizar la cotidianidad, guardar nuestros vehículos, reducir nuestro consumo desmedido de cosas, y así, la naturaleza en su sabiduría ha demostrado que en tan solo unos meses vuelve la vida a lugares donde hace décadas no se manifestaba, se aclaran las aguas, se limpia el aire, se retorna a un cierto equilibrio que la madre tierra sabe lograr. Un pequeño virus, el COVID-19 que ni siquiera podemos clasificar ciertamente como un organismo viviente, porque el debate sigue vigente y sin resolver, mientras tanto esos pequeños entes se mantienen en el limbo entre lo vivo y lo no vivo. El origen del COVID-19 aún se desconoce, sin embargo, muchas teorías apuntan a una cadena que incluye el consumo de carne de animales silvestres, los cuales son comercializados en mercados cuyas condiciones sanitarias y de inocuidad son bastante dudosas al igual que la procedencia de los especímenes consumidos en gran variedad de platos típicos, no solo en Wuhan (China), epicentro de la pandemia, sino en la mayoría de los países en desarrollo del globo terráqueo.

Pensemos por ejemplo en Colombia, aquí se consumen tortugas, armadillos, lagartos, roedores diversos, aves, peces, insectos, poniendo en riesgo no solamente la salud humana, si no también la salud de los ecosistemas al llevar al desequilibrio a poblaciones naturales cuya tasa de reproducción, o crecimiento, es menor a la de extracción por parte del ser humano; además de esto, la caza de animales silvestres usualmente conlleva también a impactos directos e indirectos sobre los ecosistemas generando así un efecto dominó que en conjunto empieza a tener efectos en escalas globales. Posiblemente el COVID-19 sea una consecuencia de muchos factores que por sí solos no tienen suficiente impacto, pero que sumados logran acumularse hasta llegar a las tragedias, como la que estamos viviendo actualmente, el calentamiento global, la contaminación, la explotación inapropiada de recursos naturales, la fragmentación de áreas naturales, el uso indiscriminado de la diversidad, entre muchos otros, están comenzando a cobrarnos factura, es momento de repensar las relaciones entre el ser humano y los factores que lo rodean.

En momentos como este, la ciencia se reivindica, todos los ojos puestos sobre los científicos y las investigaciones, con la ilusión de que muy pronto se encuentre una vacuna, un tratamiento efectivo, una forma de neutralización del virus, etc., estamos buscando una cura cuando el daño ya está hecho, sin embargo, es tiempo de pensar en las causas, y en cómo como humanidad, como comunidad nos adaptaremos a esta situación, cambiaremos paradigmas y formas de vida y por supuesto repensaremos en las formas como nos relacionamos con los animales, las plantas, los ecosistemas y los seres humanos. Ya hemos empezado, la cuarentena ha permitido la reflexión en torno a las necesidades de otros, a la importancia del autocuidado y del cuidado de los demás y por supuesto a la importancia que tienen los diferentes actores como gobernantes, centros de investigación, agricultores, el área de la salud, y tantas otras personas que sostienen y contienen la contingencia para reducir al máximo los efectos del COVID-19, no solo en la salud humana, sino también en la economía, en los patrones sociales y en todas las diversas expresiones de humanidad que se están viendo afectadas con la pandemia.

Pero volvamos al centro del objetivo de este corto escrito: las relaciones del ser humano con el entorno, concentrémonos en las relaciones con los animales, ya que ha sido el foco de atención en estos días del origen del COVID -19, pensemos por un momento todos los tipos de relaciones directas e indirectas que tenemos diariamente con los animales; al despertar nos levantamos y nos bañamos, muchos de los productos de aseo personal han sido testados en animales antes de ser comercializados, así que aquí hay una relación indirecta con los animales; nos vestimos y es probable que usemos algún cinturón de cuero, un bolso, una billetera o unos zapatos cuya materia prima proviene de un animal muerto ya sea con otros fines (consumo) o solamente por la piel (como el caso de algunos reptiles y mamíferos);

después desayunamos e incluimos usualmente productos de origen animal como leche, huevos, mantequilla, yogurt, miel entre otros (aquí tenemos una responsabilidad más explícita, lo cual no implica que sea más relevante que las anteriores); seguimos nuestro día, si tenemos mascota interactuamos con ella, la sacamos a pasear o la acicalamos un poco, tenemos un vínculo afectivo con un ser vivo de una especie diferente a la nuestra, tiene nombre, tiene un espacio emocional claro en nuestras vidas y, por supuesto, para nosotros la responsabilidad para con este ser es mucho más evidente que para con otros animales. En nuestro paseo matutino con la mascota podemos escuchar algunos pájaros en los árboles del parque, a lo mejor hasta logramos ver alguno, es fauna urbana, animales silvestres que han logrado adaptarse y convivir con nosotros, pero los pájaros no son los únicos, en los parques y zonas verdes de las ciudades habitan mamíferos como ardillas y zarigüeyas, aves diversas, reptiles muchas veces dentro de las casas, anfibios y gran variedad de insectos, y otros invertebrados; así, nuestra relación indirecta con estos animales es mucha, pues nuestro hábitat se convirtió en el de ellos y la forma como lo alteramos los afecta directamente. Sigue nuestro día, trabajamos frente al computador y en el teléfono móvil y podríamos pensar que aquí no hay ninguna relación con los animales, pero estamos muy equivocados, muchos de los materiales con los cuales se fabrican los aparatos electrónicos provienen de minería, tanto legal como ilegal, la cual tiene efectos directos sobre los ecosistemas y por supuesto sobre muchas poblaciones, usualmente afecta el agua, el suelo, el aire, desplaza especies de sus territorios naturales entre otras consecuencias, que en el caso de la minería legal se intentan mitigar con diferentes estrategias de captura y reubicación de especies, ahuyentamientos o reforestación, y así, el uso de aparatos electrónicos también tiene un efecto sobre las especies vivientes del planeta. El tiempo sigue su curso, son ahora las 11 am y debemos tomarnos una pastilla, un fármaco, ¿y esto qué tiene que ver con los animales? pues todos los medicamentos que utilizamos diariamente son probados primero en animales antes de ser usados en los seres humanos, en esta fase preclínica se emplean ratones, cobayos, conejos, y hasta perros, cerdos y por supuesto monos, que son mas cercanos a nosotros. Aquí se presenta una paradoja muy interesante: queremos una vacuna contra el COVID-19 y sin embargo no aprobamos el uso de animales en los laboratorios para las pruebas requeridas, un dilema ético bastante profundo, cuyo análisis y discusión requeriría de mucho espacio en estas páginas, así que lo dejaremos para otra ocasión. Llega la hora del almuerzo, nuevamente tenemos en el menú alimentos de origen animal pero también muchos productos de origen vegetal, ah bueno, pues estos no tienen nada que ver con los animales, ¡que alivio!, ¡Error! Muchas de las plantas que consumimos diariamente dependen de los animales para la polinización y la producción de frutos, los animales más conocidos en este proceso son las abejas, sin embargo, los animales polinizadores incluyen más de 200.000 especies entre invertebrados como las abejas, moscas, escarabajos, y vertebrados como roedores, murciélagos y aves. Así, la gratitud hacia los alimentos se la debemos tanto a los agricultores que cultivan la tierra, como a los animales que participan en el proceso, además de los productores de carne, leche y huevos.

Más allá de la cotidianidad, tenemos otras relaciones con los animales, de forma más esporádica pero igualmente impactante: pensemos en que esta persona del ejemplo se va de viaje, en su recorrido por las carreteras le ofrecen en varios puntos, loros, guacamayas, titíes, perezosos, tortugas, huevos de iguana, entre otros, los niños en su inocencia solo ven un lindo peluche vivo que quisieran llevarse a casa, el padre en su ignorancia no dimensiona los efectos negativos y termina comprando el animal por un precio monetario muy bajo que no representa en lo más mínimo el costo ambiental de sacar un animal de su hábitat, pues muchas veces son arrebatados directamente de las madres, que mueren defendiendo a sus crías, aproximadamente el 90% de los animales traficados mueren durante la captura, transporte y venta, así que los animales que logran ser vendidos (ilegalmente) solamente representa el 10 % del total de la población afectada directamente, sin contar los daños colaterales por desestructuración de las poblaciones e intervenciones ecosistémicas. Este animal que es llevado a un hogar humano, usualmente enferma y muere, por problemas nutricionales y de salud, a veces es llevado a centros de rehabilitación por rescates o entregas voluntarias, sin embargo, en muchos casos ya no cumple las condiciones para ser reintroducido a su hábitat natural y termina en una colección de zoológico o en una reserva. El viaje continúa y al llegar al destino, ofrecen diversos programas turísticos que incluyen animales: exhibiciones, cabalgatas, interacciones directas, fotografías, la mayoría de las cuales no se hacen con los mínimos criterios de bienestar para los animales, los cuales sufren, enferman y mueren en condiciones

deplorables, ¿todo para qué?, para ofrecer entretenimiento al ser humano. Así, son muchos y diversos los contextos en los cuales el ser humano tiene relaciones directas e indirectas con los animales y de esta forma las consecuencias recaen en todos: animales, humanos y ecosistemas, alterando la salud global, los órdenes y desórdenes naturales y la integridad de la vida sobre el planeta.

Pero: ¿Somos conscientes de estas relaciones que tenemos a diario con los animales? probablemente no, y por esto la presencia del COVID-19 ha ayudado a revelar, a repensar y a interiorizar muchas de estas relaciones. Esperemos que la cuarentena y la pandemia ayuden a que seamos más conscientes de las decisiones que tomamos diariamente y cómo estas traen consecuencias directas e indirectas en la supervivencia de la vida en el planeta, ya no del ser humano solamente, sino de muchas otras especies con las cuales coexistimos y con las cuales bajo el concepto de la OMS y la OIE "Una salud-Un bienestar", nosotros agregamos: una naturaleza.

Dado que el bienestar animal tiene directa relación con el bienestar humano, incluyendo la salud, es un tema actualmente prioritario, en Colombia cumplimos una década de adoptar el Bienestar animal dentro de los currículos de formación de la Zootecnia, a lo cual se sumó después la Medicina Veterinaria, también hemos avanzado en tema legislación por lo cual somos un país pionero en América latina en este tema y ciudades como Medellín son modelo de adopción de estrategias de bienestar en todos los niveles. Esto ha puesto a Medellín como sede del **Segundo congreso latinoamericano de comportamiento y bienestar animal ISAE 2020**, que inicialmente se celebraría en el mes de diciembre pero que, por la coyuntura de COVID -19, se ha postergado para 2021, esperamos que entre todos podamos hacer de este evento un espacio para la discusión de las relaciones éticas e integrales entre el ser humano y los animales en todos los aspectos: animales de granja, animales silvestres y animales de compañía, de estos últimos no hemos hablamos mucho, pero puedo decir lo siguiente: las mascotas generan vínculos emocionales fuertes con las personas con las cuales conviven; después de esta cuarentena y si llegamos a la "normalidad", esos seres usualmente de cuatro patas quedarán nuevamente solos en casa tras un largo periodo de apego al humano, ¿qué pasará por sus mentes animales? ¿qué consecuencias negativas traerá esto para ellos?, son preguntas que deberíamos empezar a hacernos. Seguramente la pandemia y la cuarentena que se generó para mitigar su impacto, dejará un terreno óptimo para la interiorización, el análisis y la propuesta de nuevos paradigmas, que incluyen por supuesto la forma de relacionarnos con todos los seres del planeta de una forma que aseguremos el bienestar global de animales, humanos y de los ecosistemas. Los invito a repensar de forma individual y colectiva cuáles relaciones tenemos a diario con los animales, qué tipo de relación es, qué consecuencias negativas puede traer tanto para los animales como para el planeta, y finalmente, qué podemos hacer al respecto, ya sea cambiando nuestra forma de consumo, eligiendo productos con bienestar animal o que aseguren la sostenibilidad o ejerciendo presión como consumidores responsables para que se cambien las formas de producción y las relaciones con los animales tengan integridad y sean éticas. Los invito a ser parte y partícipes del ISAE 2021, necesitamos amplificar la información, aumentar la conciencia de productores, estudiantes, profesionales y cualquier persona interesada en mejorar las relaciones entre humanos y animales, necesitamos hacer masa crítica para generar realmente cambios significativos. La pandemia de COVID-19 debería generar el cambio radical de la humanidad tal y como ocurrió con la peste negra, las guerras y hambrunas del siglo XIV que contribuyeron al fin del medioevo y al inicio del renacimiento en el siglo XV. Una nueva humanidad se está gestando en esta pandemia, seamos parte del nacimiento del nuevo ser humano consciente siglo XXI.

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Expected genetic gains from mono trait and index-based selection in advanced bread wheat (*Triticum aestivum* L.) populations

Ganancia genética esperada en la selección basada en rasgos únicos e índices en una población avanzada de trigo pan (*Triticum aestivum* L.)

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ABSTRACT

This study aimed at evaluating the expected gains from selection obtained based upon direct, indirect, and index-based selection in a set of 599 bread wheat lines. The experiment was carried out at the experimental field of INRAA institute, Setif research unit (Algeria), in a Federer augmented block design including three controls. A wide range of genetic variability was observed among lines for the eleven traits assessed. The results indicated that index-based selection and selection based on grain yield expressed higher expected genetic gain than direct and indirect mono-trait-based selection. The best 15 selected lines exhibited higher grain yield than the control varieties, and they were clustered in three groups that contrasted mainly for the flag-leaf area, thousand-kernel weight, biomass, and harvest index. The index-based selection appears as a useful tool for the rapid selection of early filial generations, enriching selected breeding materials with desirable alleles and reducing the number of years required to combine these traits in elite varieties.

Keywords:
Drought
Genetic gain
Index
Simultaneous selection
Yield

RESUMEN

Este estudio investigó los beneficios esperados de la selección obtenida en base al índice directo, indirecto y basado en la selección en un conjunto de 599 líneas de trigo panadero. El experimento se llevó a cabo en el campo experimental del instituto INRAA, unidad de investigación de Setif (Argelia), en un diseño de bloques aumentados Federer que incluye tres controles. Se observó un gran rango de variabilidad genética entre las líneas para los once rasgos evaluados. Los resultados indicaron que la selección basada en índices y la selección basada en el rendimiento de grano expresaron una mayor ganancia genética esperada que la selección directa e indirecta basada en rasgos individuales. Las 15 primeras líneas seleccionadas exhibieron un mayor rendimiento de grano que los controles y se agruparon en tres grupos que divergieron de manera eficiente para el área de la hoja bandera, el peso de mil granos, la biomasa, el rendimiento de grano y el índice de cosecha. La selección basada en índices aparece como una herramienta útil para la selección rápida de las primeras generaciones filiales; enriqueciendo materiales de reproducción seleccionados con alelos deseables y reduciendo el número de años necesarios para combinar estos rasgos en variedades de élite.

Palabras clave:
Sequía
Ganancia genética
Índice
Selección simultánea
Rendimiento

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In cereal crops, artificial selection has rapidly evolved, covering a wide range of technical and scientific activities, with the objective of developing varieties, with high yield potential, good adaptation, and end-use quality. Selection can be mono or multi-traits, early or late. Mono-trait selection is based on simply inherited traits such as earliness, plant height, and disease resistance. It is usually carried out early on F_2, F_3 up to F_4 filial generations. For complex traits such as yield, accumulated biomass, and tillering capacity, it is practiced in advanced generations that are somehow fixed and have been subjected to single-trait selection in early generations for highly inherited traits. Selection effectiveness is largely dependent on the existing genetic variability among individuals making up the population under selection. Only the genetic part of the variability is transmissible to the offspring and is useful for selection. Selection efficiency is also dependent on the degree of genetic determination, which measures the proportion of genetic variability within the total phenotypic variability (Acquaah, 2007). Heritability represents the breeder's ability to recognize the difference between individuals for a given trait and, therefore, the possibilities to improve this trait. High heritability values allow breeders to have a grip on the selected trait (Fellahi *et al.*, 2017). In addition to phenotypic variability and heritability, selection efficiency also depends on the relationships among the traits. A better understanding of the existing relationships between traits is interesting and necessary to identify those which could serve as selection criteria and what are their effects on traits not targeted in the selection process. Yield is a complex quantitative trait whose variation is under polygenic control, predominantly of non-additive nature. It exhibits low heritability, instability and presents significant genotype×environment interaction (Purshase *et al.*, 2000; Annicchiarico *et al.*, 2005; Montesinos-López *et al.*, 2018, Laala *et al.*, 2018). Look for alternative methods and selection criteria to improve this trait efficiently is necessary (Bouzerzour, 1998). Indirect selection is an analytical method based on the use of morpho-physiological traits and yield components that are predictive of yield potential and good abiotic stress tolerance (Fleury *et al.*, 2010; Reynolds and Langridge, 2016). Kumar and Bahl (1992) noted that indirect selection might be more efficient when the secondary trait is highly correlated with the primary trait (usually grain yield) and is easily measurable. This method involves several steps ensuring enough knowledge of the physiological mechanisms of drought tolerance followed by the genetic study of these traits before moving to their use as selection criteria in the breeding scheme. Index-based selection is currently used to make the selection more efficient (Carvalho *et al.*, 1999). The index is, by itself, an additional trait, established through an optimal combination of several measured characteristics, for which simultaneous selection is sought, to improve overall population phenotypic values (Cruz *et al.*, 2006). Several indices were developed, and are currently employed in various cultivated crops such as wheat (Ghaed-Rahimi *et al.*, 2017; Fellahi *et al.*, 2018), cotton (Ribeiro *et al.*, 2018), popcorn (Lima *et al.*, 2018), snap bean (Gomes *et al.*, 2018), soybean (Bizari *et al.*, 2017), potato (Silva G *et al.*, 2018a), sugarcane (de Azeredo *et al.*, 2017), and strawberry (Vieira *et al.*, 2017). The use of index-based selection in genetic improvement of field crops helps breeders for selecting genotypes that combine high yield potential and other desirable characteristics. This research aims at investigating the variation in expected genetic gains generated in response to mono-traits and index-based selection and to compare the efficiency of these selection strategies in advanced bread wheat (*Triticum aestivum* L.) populations.

MATERIALS AND METHODS

Plant materials and experimental design

The experiment counted with 602 genotypes, including 599 F_4-breeding lines and three standard varieties. Breeding lines were developed through pedigree selection method, from 20 bi-parental crosses made between nine bread wheat genotypes. Controls were Rmada (Vee's/Bow's// Alondra's/Pavon's), Hidhab (HD1220/3*Kal/Nac), and El-Wifak (K134/4/Tob/ Bman/Bb/3/Cal/5/Bucc), which are registered as varieties. Plant material was grown, during the 2014-2015 cropping season, in the experimental field of the Algerian National Institute of Agronomic Research (INRAA), Research Unit of Setif (INRAA-UR Setif, 36°15'N; 5°87'E; 1,081 masl). The experiment was arranged in a Federer augmented block design (Federer, 1955). Each of the three blocks contained 200 breeding lines and three controls. The experimental plot is a single 1-m long row, spaced 0.2 m apart, having 0.2 m^2 area. Land preparation was carried out according to the recommended practices for the area. Before sowing, 100 kg ha^{-1} of 46%

superphosphate fertilizer was applied. Granstar [Tribenuron methyl] herbicide was used to control weeds, and, when necessary, weeds were removed manually to keep the crop clean. At the tillering stage, plots were fertilized with 75 kg ha^{-1} of 35% urea. As April rainfall was almost nil (6.8 mm), and to avoid crop failure, the experiment was irrigated thrice during the May –June period, adding 60.0 mm irrigation water to the 343.6 mm rainfall received during the crop cycle (September-June).

Measurements

The following traits were assessed: Flag leaf chlorophyll content (CHL, Spad) was measured at the heading stage with SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). Canopy temperature (CT, °C) was determined at the same growth stage in each plot, between 11:00 h and 14:00 h, using a Sixth Sense LT300 infrared thermometer, following the procedure described by Pask *et al.* (2012). Flag leaf area (FLA, cm^2) was determined according to the procedure established by Spagnoletti-Zeuli and Qualset (1990) as **$FLA(cm^2)=L(cm)xl(cm)x0.607$**, where L and l refer to the flag leaf length and width, respectively; 0.607 is the regression coefficient relating leaf area determined by the gravimetric method to leaf area determined by the L×l product. Heading date (HD, d) was recorded as the number of calendar days from January first to the date when 50% of the spikes were half-way out from the flag leaf sheath. Plant height (PH, cm) was measured at maturity from ground level to terminal spikelet tip, awns excluded. Above-ground biomass (BIO, g m^{-2}) was estimated from a hand-harvested area of one row, 0.5 m long×0.2 m inter-row spacing. This sample also served, after mechanical threshing, to determine grain yield (GY, g m^{-2}) and the number of spikes (SN, # of spikes per m^2). Thousand kernel weight (TKW, g) was derived from the count and weight of a seed sample of 250 grains. The number of grains per spike (NGS, #) was derived from estimated values of grain yield, number of spikes and thousand kernel weight as **$NGS=NGP/SN$** where: NGP is the number of grains per plant, calculated by the equation: **$NGP=1000(GY/TKW)$**. Harvest index (HI, %) was obtained by the formula: **$HI(\%)=100(GY/BIO)$.**

Statistical analysis

Data collected were subjected to an analysis of variance (ANOVA) following the procedure outlined by Cruz (2006). The linear model for the analysis of the augmented block is: $\mathbf{Y_{ij}=\mu+c_i+g_{ij}+\beta_j+\varepsilon_{ij}}$ where: Y_{ij} is the treat mean value for the i^{th} treatment, which can be broken down into c_i which is the effect of the i^{th} control and g_{ij} which is the effect of the i^{th} genotype in the j^{th} block; β_j is the effect of the j^{th} block and ε_{ij} is the residual associated with the i^{th} treatment in the j^{th} block. Fisher's least significant difference test at 5% probability level ($LSD_{5\%}$) was calculated according to Steel and Torrie (1960). The phenotypic (σ^2_p), genotypic (σ^2_g), and environmental (σ^2_e) variances were calculated for each trait based on the expected mean squares derived from the ANOVA table. These variances served to calculate genetic and environmental parameters, including the variation index, which is the ratio $\mathbf{Cv_g/Cv_e}$, where Cv_g is the genetic coefficient of variation, and Cv_e is the residual coefficient of variation. Broad-sense heritability was calculated according to Cruz (2006)**:** $\mathbf{H^2_{bs}=100(\sigma^2_g/\sigma^2_p)}$ where: σ^2_g and σ^2_p as defined above.

Selection strategies and expected genetic gains

Three selection approaches were employed to estimate direct and indirect expected genetic gains among the F_4 lines displayed through mono-trait and indices-based selection. Direct selection expected gain was calculated according to Cruz (2006): $\mathbf{\Delta G_i=H_i^2xS_i=H^2_ix(\bar{X}_{si}-\bar{X}_{0i})}$ where ΔG_i refers to the expected gain, H^2_i is the broad-sense heritability; S_i is the selection differential, $\bar{X}_{si}$ is the mean value of the selected lines and $\bar{X}_{0i}$ is the mean estimate of the base population for the i^{th} trait. Direct selection gain, was expressed as the percentage of the population mean, as follow: $\mathbf{\Delta G_i\% = (\Delta G_i x100)/\bar{X}_{0i}}$. Selection was done in the sense of increased values for each one of the measured traits, except for canopy temperature and days to heading for which decreasing mean value was targeted. Indirect selection gain was estimated as outlined by Cruz (2006): $\mathbf{GS_{j(i)}=H^2_jx(\bar{X}_i-\bar{X}_{0j})=H^2_ixDS_{j(i)}}$, where: $GS_{j(i)}$ refers to the j^{th} trait gain, when the selection was based on the i^{th} trait; $\bar{X}_{j(i)}$ is the mean value of the j^{th} trait for the selected lines based on the i^{th} trait; $\bar{X}_{0j}$ is the mean value of the j^{th} trait; $\bar{X}^2_j$ is the broad-sense heritability of the j^{th} trait and is to the selection differential of the j^{th} trait, in which the selected lines showed the best performance for the i^{th} trait. Indirect selection gain is converted as the percentage of the population mean as follow: $\mathbf{GS_{j(i)}\%=(GS_{j(i)}x100)/\bar{X}_{0i}}$. Expected gain was also calculated using Smith and Hazel (1943), Williams (1962), Pesek and Baker (1971),

Subandi *et al.* (1973), Mulamba, and Mock (1978) and Cruz (2006) indexes. A value of one was adopted as the economic weight for all selection strategies applied. Among the 599 evaluated lines, the top15 breeding lines, for each selection approach, were identified. The coincidence coefficient was obtained through the ratio of double coincident lines between two selection approaches and the total of selected lines, in percentage. A high coincidence coefficient, between two selection approaches, indicated concordance of their results (Gomes *et al.*, 2018). Statistical data analysis was done using Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

Genetic variability and heritability

Even though the overall mean somewhat hid variability present among breeding lines, results of the analysis of variance revealed a significant genotype effect for plant height, above-ground biomass, grain yield, and harvest index at a 5% probability level. The residual coefficient of variation values (Cv_e) was below 17%, indicating a relatively good experimental precision (Table 1). Comparisons of range magnitude with critical value for yield components, and related agronomic traits, provide evidence of the presence of appreciable genetic variability among the breeding lines, suggesting that selection within these populations could lead to the identification of potentially useful breeding lines. Chlorophyll content varied from 33.5 to 58.3 Spad, canopy temperature ranged from 20.4 to 29.9 °C, heading date from 125.0 to 139 days, and flag leaf area from 7.3 to 42.7 cm. Plant height ranged from 45.0 to 121.0 cm, the number of spikes from 110.0 to 920.0 spikes per m^2, while thousand-kernel weight varied from 30.0 to 61.0 g and the number of grains per spike from 10.8 to 63.6 grains (Table 1). Above-ground biomass ranged from 320.0 to 3,440.0 g m^{-2}, grain yield from 102.0 to 1,434.0 g m^{-2} and harvest index from 14 and 61.3%.

Table 1. Analysis of variance results, overall mean, range, least significant difference ($LSD_{5\%}$), broad-sense heritability (H^2_{bs}), coefficients of genetic (Cv_g), and experimental (Cv_e) variation and the ratio of genetic to the residual coefficient of variation (Cv_g/Cv_e) for the measured traits.

Traits	Source of variation		Cv_e (%)	Overall mean	Range		$LSD_{5\%}$	H^2_{bs} (%)	Cv_g (%)	Cv_g/Cv_e
	Treatment (601)	Error (4)			min	max				
CHL	14.1	11.2	7.7	43.5	33.5	58.3	5.4	23.7	4.3	0.6
CT	3.8	3.7	7.6	25.1	20.4	29.9	3.1	24.0	4.3	0.6
HD	3.9	1.6	1.0	127.6	125	139	2.0	79.5	2.0	2.0
FIA	30.3	10.2	16.5	19.4	7.3	42.7	5.1	77.4	30.6	1.9
PH	95.0 *	15.4	5.1	76.9	45.0	121.0	6.3	86.5	12.9	2.5
SN	17,391.4	7,466.7	16.9	510.5	110	920.0	138.6	56.8	19.5	1.1
TKW	20.1	3.8	4.2	46.3	30.0	61.0	3.1	91.8	14.1	3.4
NGS	27.8	12.7	12.6	28.2	10.8	63.6	5.7	80.3	25.5	2.0
BIO	236,652.1 *	22,377.8	9.7	1,536.4	320	3,440.0	239.9	91.0	31.0	3.2
GY	51,580.9 **	2,941.4	8.1	667.5	102	1,434.0	87.0	94.8	34.9	4.3
HI	31.1 *	4.7	5.0	43.4	14	61.3	3.5	85.9	12.3	2.5

CHL: Chlorophyll content (Spad), CT: Canopy temperature (°C), HD: Heading date (d), FLA: Flag leaf area (cm^2), PH: Plant height (cm), SN: Number of spikes, TKW: Thousand kernel weight (g), NGS: Number of grains per spike, BIO: Above ground biomass (g m^{-2}), GY: Grain yield (g m^{-2}), HI: Harvest index (%). * and **: significant effect at 5% and 1% probability, respectively.

Broad-sense heritability estimates varied from 23.7 to 94.8% (Table 2). High H^2_{bs} values were recorded for grain yield (94.8%), thousand kernel weight (91.9%), biomass (91.0%), plant height (86.50%), harvest index (85.9%), number of grains per spike (80.3%), heading date (79.5%), flag leaf area (77.4%), and number of spikes (56.8%). This behavior suggests that the traits mentioned above are highly heritable, being less subjected to environmental variation. This is supported by the Cv_g/Cv_e, ratio values which are greater than one, suggesting a

good correlation between predicted and observed mean values for these traits, and favorable selection conditions for achieving high genetic gains. Chlorophyll content (23.7%) and canopy temperature (24.0%) showed low $H^2_{b\ s}$ estimates associated with Cv_g/Cv_e ratio values below unity, indicating that both traits were subjected to a significant environmental effect. Genetic coefficient of variation (Cv_g) values varied from 2.0% to 34.9%. High values were observed for flag leaf area (30.6%), spikes fertility (25.5%), biomass (31.0%), and grain yield (34.9%); indicating that these traits showed enough variability, which could be exploited through selection.

Expected genetic gains

Expected genetic gains, expressed as a percent of the base population mean, are presented in Table 2. Total expected genetic gains varied from -19.6% for selection based on the number of days to heading to 248.0% for selection based on the Subandi index. Expected genetic gains exhibited by direct selection for CHL, CT, and HD were lower than 5.0% for CHL (4.9%), CT (-4.2%), and HD (-3.1%, Table 2). CHL selection improved FLA indirectly by 10.7%, HI by 8.4%, and reduced BIO by -8.6%. CT selection caused significant improvement in FLA, SN, TKW, BIO, and GY, which increased by 12.2, 13.7, 11.1, 32.3, and 30.7%, respectively. HD selection induced significant changes in TKW, which gained 9.0%, NGS, FLA, and BIO, which decreased by 14.9, 5.7, and 5.7%, respectively. CH, CT, and HD expected genetic gains derived from indirect selection for FLA, PH, SN, NGS, BIO, GY, and HI were almost nil, being less than 2.0% and were lower than expected genetic gains caused by direct selection (Table 2). CHL and CT have low broad-sense heritability, while HD showed relatively weak variability, as suggested by its low Cv_g value (Table 1). These results corroborated with those of Bárbaro *et al.* (2007), who found that larger gains are generated from characters exhibiting greater genetic variation while lower gains are expected from selection for traits

Table 2. Estimates of expected genetic gain as a percentage of base population mean, generated from direct and indirect selections based on individual measured traits and indices.

Selection criterion	Traits											Sum
	CHL	CT	HD	FLA	PH	SN	TKW	NGS	BIO	GY	HI	
CHL	4.90	1.20	-0.40	10.70	-0.20	-1.10	2.30	-1.30	-8.60	-1.30	8.40	14.50
CT	0.50	-4.20	0.30	12.20	4.30	13.70	11.10	-4.70	32.30	30.70	-2.00	94.20
HD	-0.10	-0.60	-3.10	-5.70	-2.90	3.20	9.00	-14.90	-5.70	-1.70	2.90	-19.60
FLA	-0.10	-0.60	0.60	70.10	11.00	3.30	1.90	8.30	17.30	17.20	-0.30	128.60
PH	-0.80	0.40	1.40	17.10	28.90	2.30	0.80	4.00	20.80	7.70	-9.20	73.40
SN	0.40	-1.00	-0.60	-0.70	8.40	37.20	4.20	-1.30	60.10	65.60	2.20	174.60
TKW	-0.20	-1.20	-1.70	-1.40	0.20	2.40	30.00	-28.70	1.30	-1.70	-2.50	-3.40
NGS	0.00	0.60	1.40	-11.80	-0.80	1.70	-7.40	49.00	37.20	48.40	6.30	124.60
BIO	0.40	-1.50	1.90	10.50	8.60	29.50	1.20	19.30	81.40	80.40	-1.20	230.60
GY	0.60	-1.50	0.80	4.90	4.30	28.80	-0.80	27.30	73.60	88.90	7.40	234.30
HI	1.70	-1.70	-0.40	8.80	-4.10	-1.90	4.10	11.90	-10.30	13.40	27.80	49.10
SHI	0.70	-1.70	0.70	5.90	4.40	26.90	1.20	26.30	72.00	87.30	7.30	230.90
MMI	2.00	-0.50	1.00	39.80	6.80	16.00	-0.10	25.50	43.10	61.90	11.90	207.70
WI	0.40	-1.30	1.40	6.20	8.60	30.60	-0.40	23.20	79.80	85.50	2.60	236.70
SI	1.30	-1.70	1.50	45.70	8.30	23.60	-0.70	26.10	59.50	75.30	9.10	248.00
PBI	1.50	1.70	1.40	27.40	22.60	-0.80	6.80	6.10	15.00	11.50	-2.70	90.50
CI	2.00	-1.50	1.40	47.50	8.20	18.50	-0.40	26.90	49.10	66.80	11.10	229.60

CHL: Chlorophyll content (Spad), CT: Canopy temperature (°C), HD: Heading date (d), FLA: Flag leaf area (cm^2), PH: Plant height (cm), SN: Number of spikes, TKW: Thousand kernel weight (g), NGS: Number of grains per spike, BIO: Above ground biomass (g m^{-2}), GY: Grain yield (g m^{-2}), HI: Harvest index (%). ISH: Smith & Hazel Index, MMI: Mulamba & Mock Index, WI: Williams Index, SI: Subandi Index, PBI: Pesek & Backer Index, CI: Cruz Index. Sum: Total expected genetic gain per selection criterion.

showing lower genetic variability. Based on the mono-trait selection and among the three traits cited above, CT, with total gains of 94.2%, comes out as a surrogate trait to improve indirectly and simultaneously FLA, SN, TKW, BIO and GY without any significant changes for the other measured traits. Mono trait selection for any one of the following variables FLA, PH, SN, NGS, TKW, BIO, GY, and HI, generated higher direct expected genetic gains than indirect selection. In fact, the expected genetic gains from direct selection against the best expected genetic gains of indirect selection were 70.1% vs 47.5% via CI, for FLA; 28.9% vs 22.6% via PBI for PH; 37.2% vs 30.6% via WI for SN; 30.0% vs 11.1% via CT for TKW; 49.0% vs 27.3% via GY for NGS; 81.4% vs 79.8% via WI for BIO; 88.9% vs 87.3% via WI for GY; and 27.8% vs 11.1% via CI for HI (Table 2). These results corroborated those reported by Bizari *et al.* (2017), who found that gains from direct selection were superior to those for indirect gains, for all the characters. Cargnin *et al.* (2007) compared three selection indices in 240 $F_{2:4}$ wheat families derived from eight segregating wheat populations and reported that the highest gains were estimated by direct selection.

The advantage of multi-trait selection is analyzed based on total expected genetic gains. As total gains obtained from SHI, MMI, WI, SI, and CI selection indices were of the same magnitude as those generated through selection based on GY (Table 2).

Therefore, the comparison of mono vs. multi-trait selection efficiency was made relatively to selection based on GY. Analysis of indirect gains of mono-trait selection based on CHL, CT, HD, and FLA relative to GY based selection showed a strong negative effect of this selection strategy on GY, SN, NGS, and BIO (Figure 1). In fact, 49.0, 32.0, 49.0, and 39.0% reduction in GY expected genetic gain was observed when mono trait selection was based on CHL, CT, HD, and FLA, respectively. Observed declines in BIO expected genetic gains were 50.0, 25.0, 48.0 and 34.0% for the same selection criteria; NGS expected genetic gain was reduced also by 27.0, 30.0, 39.0 and 18.0%; while SN expected gain declined by 35.0, 18.0, 30.0, and 30.0%, respectively. These decreases indicated the inefficiency of these traits as a selection criterion, relatively to GY selection (Figure 1).

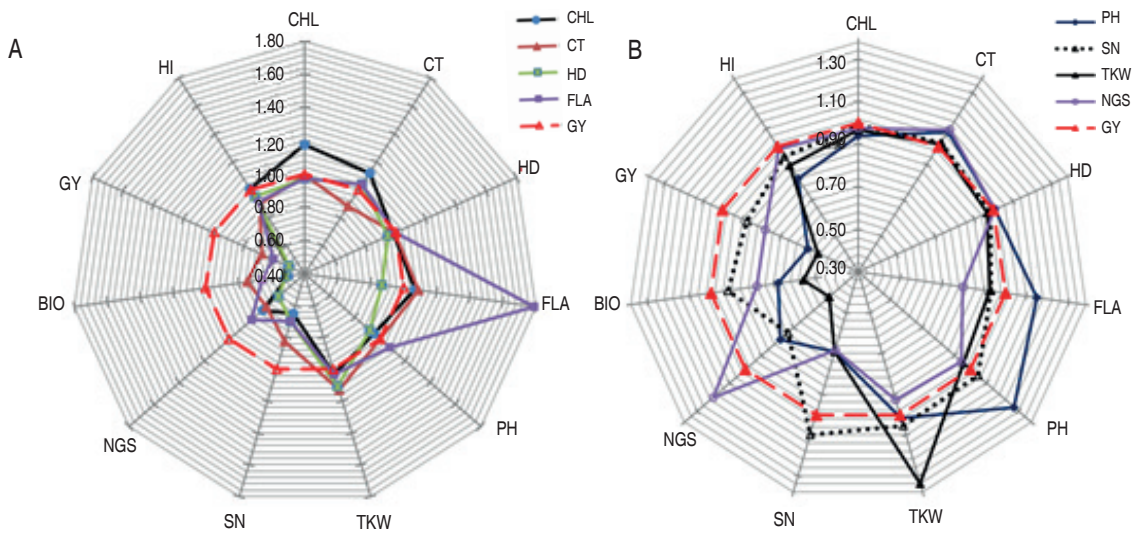

Figure 1. Direct and indirect gains of mono-trait selection based on A. CHL, CT, HD, and FLA; B. based on PH, SN, TKW, NGS, relatively to GY based selection.

Besides, their direct gain which was greater (27.0%, 10.0%, 34.0%, and 20.0% for PH, SN, TKW, and NGS, respectively) than indirect gain due to GY based selection, analysis of indirect gains exhibited by these traits indicated a sizeable negative effect on GY, BIO, and HI (Figure 1). Indirect gain due to selection based on PH declined GY, BIO, and HI by 44.0, 32.0, and 18.0%, respectively, and caused 31.0 and 22.0% reduction in NGS and NS, respectively. The indirect gain for TKW, caused by PH based selection was almost zero.

Selection based on SN declined GY, BIO, and HI by 13.0, 8.0, and 6.0 %, respectively, and caused a 27.0% reduction in NGS. Indirect gains for PH and TKW, due to SN based selection, were below 5.0%. Selection based on TKW declined GY, BIO, and HI by 49.0, 44.0, and 11.0%, respectively, and caused a 52.0% reduction in NGS and 31.0% in SN (Figure 1). The indirect gain for PH due to TKW based selection, was low, having a 4.0% value. Selection based on NGS declined GY and BIO by 22.0% and caused a 32.0% reduction in SN and 7.0% in TKW. Indirect gains for HI, PH, and TKW from indirect selection based on NGS were 1.0, 6.0, and 7.0%, respectively (Figure 1). Because of negative indirect expected genetic gains, selection based on these traits appears to be inefficient when compared to indirect gains induced through GY based selection. Selection based on BIO resulted in almost similar direct and indirect gains as GY based selection, but HI based selection caused a significant gain decrease in NGS (14.0%), SN (36.0%), BIO (0.51.0%), and GY (41.0%, Figure 2). Analysis of the effect of index-based selection suggested that SHI and WI expressed equivalent results as GY based selection. Compared to GY based selection, MMI, SI, PBI, and CI index-based selection caused significant indirect gain increases, 20.0% to 52.0%, in FLA. MMI induced 15.0, 16.0, and 15.0% gain a decrease in SN, BIO, and GY, respectively. SI caused gain decrease value of 9.0 and 7% for BIO and GY while application of PBI appeared as the least efficient selection strategy among index-based selection, as it induced negative indirect gains in NGS (210%), SN (34.0%), BIO (36.0%), GY (42.0%) and HI (10.0%). CI-based selection, compared to GY based selection, decreased indirect genetic gain in SN (12.0%), BIO (15.0%), and GY (12.0%, Figure 2).

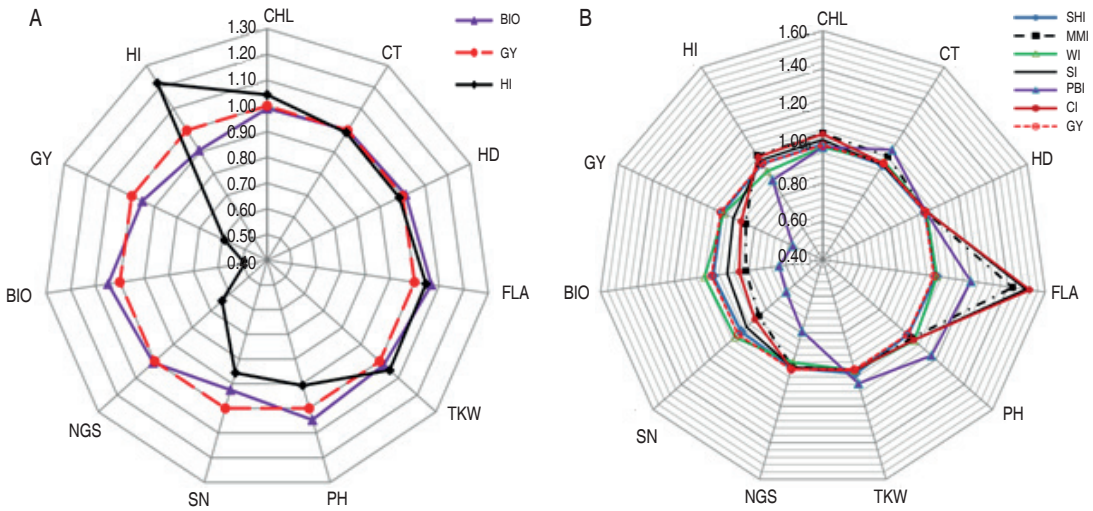

Figure 2. Direct and indirect gains of mono-trait selection based on A. BIO and HI; B. based on indices, relatively to GY based selection.

Globally, it can be said that index-based selection appeared to be more efficient than individual trait-based selection, but equivalent to GY based selection. In this context, Costa *et al.* (2008) reported that SHI and PBI indices were advantageous over direct selection for soybean grain yield. Index-based selection generated gains that are evenly distributed among all traits. This is useful since, in stressed environments, genotypes, bearing a combination of physiological and morphological traits known to influence positively plant performance, are desirable. In this study, SHI and WI expressed equivalent results as GY based selection, with positive gains for all traits included in the index. Using index-based selection Silva and Viana (2012), as well as Vasconcelos *et al.* (2010), found greater and well-distributed gains for the main characters of the passion fruit and alfalfa. In this study, the PBI index was the least efficient among the six evaluated indices. This index resulted in genetic variation loss in several important desired traits, exhibiting negative indirect gains. This contrasted with the results of Cargnin *et al.* (2007), who demonstrated that PBI exhibited the highest expected genetic gains. These authors reported that gains expected through indices selection for

grain yield were larger than those obtained by the direct and indirect mono-trait selection, except for yield-based selection. Mahdy (1988) found that the selection-based index was more efficient for yield improvement compared to single-trait selection in a segregating population of wheat. Silva B *et al.* (2018) found that SHI provided high predicted gains for all the assessed traits in elephant grass. Ribeiro *et al.* (2018) found that MMI was the most suitable index for selecting high yielding genotypes with good fiber technological components in upland cotton. Bhering *et al.* (2012) reported that SI was promising for selection, providing balanced predicted gains for several traits of interest. According to Valério *et al.* (2009), as well as Fellahi *et al.* (2018), differences in the results of direct, indirect selection and index-based selection depend on crosses background, targeted selected traits and genotype×environment interaction.

The results of the present study indicated, so far, that SHI and WI index-based selection was advantageous to obtain sizeable gains distributed among several desired traits, without significant gain loss in the principal trait. This selection strategy is useful when a breeder focused on developing plant ideotype. The results indicated that high expected genetic gains were observed from single trait selection. This is useful to identify genotypes carrying interesting genes, like those related to stress tolerance (i.e., canopy temperature), which are often weakly associated with the principal trait (i.e., grain yield). Such genotypes could be used in biparental crosses to increase genetic variability and to accumulate favorable genes in elite advanced breeding lines. The coefficients of coincidence values ranged from 0 to 86.7%, being low for direct selection based on CHL, HD, FLA, and PH, which resulted in few lines selected simultaneously, and high for yield, yield components, and index-based selections, excluding PBI index. None of the lines selected for GY were found among those selected for CHL, HD, FLA, PH, and PBI. The highest coincidence coefficients were recorded for WI (86.7%), SHI (80.0%), and BIO (66.7%), suggesting outcomes likeness of selection based on GY, SHI, WI and to lesser extend on BIO.

Characterization of the potentially high yielding breeding lines

In order to identify the most promising genotypes for grain yield, all the selected lines through different methods were scored according to their appearance frequency. The scoring results indicated an absolute frequency ranging from one to eight. The top 15 breeding lines (Lines number 11, 55, 59, 60, 99, 150, 159, 160, 180, 182, 189, 285, 300, 521, and 558), with a score equal or greater than 4, were retained. These lines descended from nine out of twenty bi-parental populations studied by Fellahi *et al.* (2018). Compared to the average of the control varieties, the relative changes brought by the top 15 lines are shown in Figure 3. Significant improvements were achieved for FLA (26.22%), PH (14.62%), NGS (20.31%), BIO (38.99%), GY (44.04%), and CT (-14.53%). These differences were 1 to 4 times higher than $LSD_{5\%}$. These results indicated that the effective selection of superior

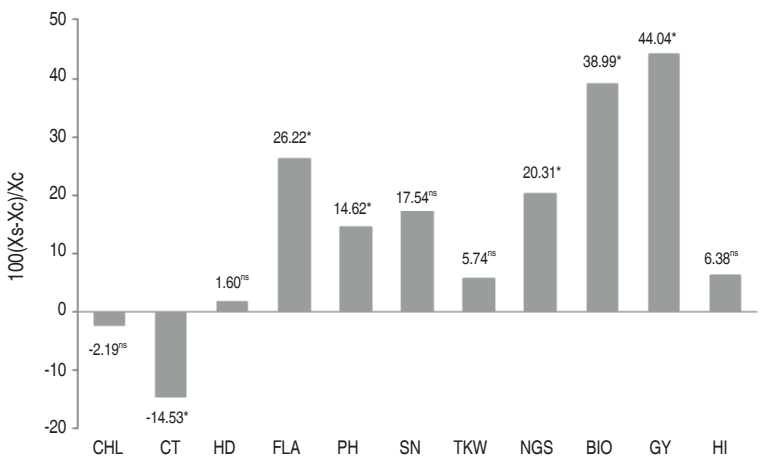

Figure 3. Relative deviation of the average of the 15 retained lines (X_S) from the average (X_C) of the three control varieties [$100\times(X_S-X_C)/X_C$)] for the assessed traits.

individuals within this plant material contributes certainly to the improvement of yield and its related traits under conditions in the semi-arid regions of the Algerian high plateaus. Based on the Euclidian distance, Ward's method, these breeding lines formed three clusters (Figure 4). Lines 11, 59, 90, 150, 180, and 558 clustered apart (cluster 1). Lines 55, 99, and 182 formed cluster 2; while lines 160, 285, 521, 159, 189, and 300 grouped in cluster 3. The average values per cluster (absolute value and expressed as % of maximal value) indicated that FLA, BIO, GY, and TKW and HI discriminated efficiently between clusters (Figure 5, Table 3).

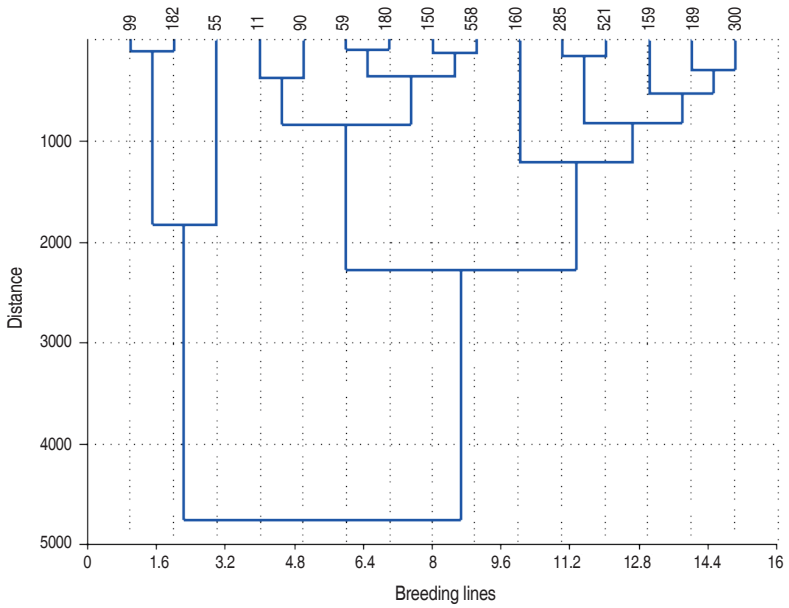

Figure 4. Genotypes clustering based on the eleven measured traits in the top 15 selected lines.

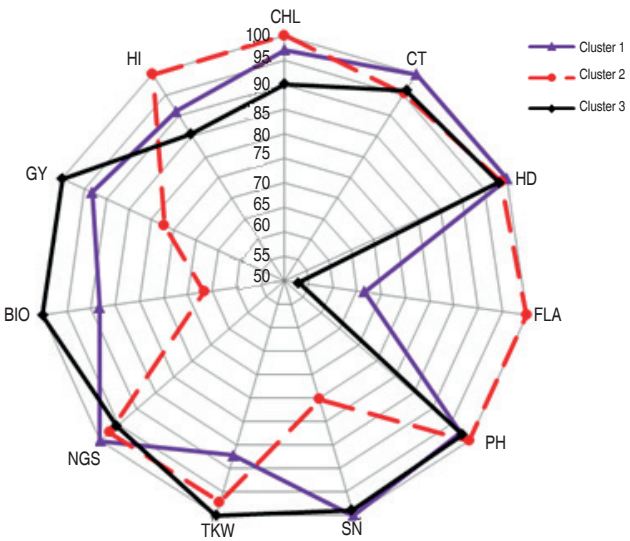

Figure 5. Behavior of the three clusters for the eleven measured traits in the top 15 selected lines.

Table 3. Cluster average values for the measured traits.

Clusters	CHL	CT	HD	FLA	PH	SN	TKW	NGS	BIO	GY	HI
1	46.4	23.6	130.9	26.4	81.8	774.4	43.9	37.6	2667.8	1242.4	47.2
2	47.8	22.5	129.2	39.7	83.9	583.3	48.9	36.6	2014.4	1024.9	51.8
3	43.1	22.7	128.5	21.0	82.3	765.6	50.3	35.9	3023.3	1330.8	44.3

CHL: Chlorophyll content (Spad), CT: Canopy temperature (°C), HD: Heading date (days), FLA: Flag leaf area (cm^2), PH: Plant height (cm), SN: Number of spikes, TKW: Thousand kernel weight (g), NGS: Number of grains per spike, BIO: Above ground biomass (g m^{-2}), GY: Grain yield (g m^{-2}), HI: Harvest index (%).

Genotypes belonging to cluster 3 had the best values in terms of yield performance, biomass and grain weight with a short vegetative cycle, low chlorophyll content, small leaves, and lower spike fertility compared to the genotypes of clusters 1 and 2. Lines of cluster 2, which had larger leaves with high chlorophyll content and decreased canopy temperature, were taller, less performing with increased harvest index. On the other hand, genotypes of cluster 1 were later, shorter with increased canopy temperature and fertile tillers. They were also characterized by low grain weight compared to the genotypes of clusters 2 and 3 (Figure 5).

CONCLUSION

Heritability estimates were sufficient for the prediction of next-generation means when the plant material selected is under the same environmental and management conditions. The results pointed out that those high expected genetic gains were observed from single trait selection, suggesting possibilities to identify genotypes carrying interesting genes, which are often weakly associated with grain yield. However, index-based selection was more efficient over the above approaches employed. Indeed, Smith and Hazel index and William index expressed equivalent results as grain yield based selection and provided the most satisfactory gains in all traits studied. The coefficients of coincidence values were low for direct selection based on chlorophyll, heading date, flag leaf area, and plant height, which resulted in few lines selected simultaneously, and high for yield, yield components, and index-based selections, excluding Pesek and Baker index. Cluster analysis grouped the 15 best-selected lines in three divergent groups that discriminated clearly for flag leaf area, thousand kernel weight, biomass, and harvest index.

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Agronomic evaluation of biofortified beans in Antioquia producers' farms

Evaluación agronómica de frijoles bio-fortificados en fincas de agricultores de Antioquia

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ABSTRACT

The objective of this research was to evaluate genotypes of iron- and zinc-enriched common beans during breeding in producers' farms. Yield, disease reaction, and commercial grain characteristics were evaluated to achieve this objective. In three locations of Antioquia (Rionegro, Jardín, and Betulia), seven bush beans and eight climbing bean genotypes were planted. A randomized complete block design with four replications was used in each location. There were significant differences between the bush and climbing bean genotypes that were evaluated. The highest yields, in all locations, were for the biofortified bean NUA 45 and the control variety Uribe Rosado, followed by the CAL 96 and AFR 612 genotypes. For the climbing beans, the highest yields were found in the G2333 genotypes, being this treatment equal to the MAC 27, a bean that is adapted to mid-climate and altitudes. The MAC 27 material is presented as a promising variety because of its high yields and tolerance to diseases, mainly anthracnose.

Keywords:
Human diet
Iron
Micronutrients
Phaseolus vulgaris L.
Zinc

RESUMEN

El objetivo de esta investigación fue evaluar genotipos de fríjol común enriquecidos con hierro y zinc en fincas de productores. Para alcanzar este propósito se evaluó el rendimiento, la reacción a enfermedades y características comerciales del grano. En tres localidades (Rionegro, Jardín y Betulia) de Antioquia se sembraron siete genotipos arbustivos y ocho genotipos volubles de fríjol. Se utilizó el diseño de bloques completos al azar con cuatro repeticiones en cada localidad. Se presentaron diferencias significativas, entre los materiales arbustivos y volubles evaluados. Los mayores rendimientos en todas las localidades se presentaron para el material bio-fortificado NUA 45 y el control Uribe Rosado, le sigue en su orden los genotipos CAL 96 y AFR 612. Para los materiales volubles los mayores rendimientos se presentaron para el material G2333, este tratamiento fue igual al genotipo MAC 27, un frijol adaptado a clima y altitud medio. Se presenta el material MAC 27, como promisorio por sus altos rendimientos y tolerancia a enfermedades, principalmente a la antracnosis.

Palabras clave:
Dieta Humana
Hierro
Micronutrientes
Phaseolus vulgaris L.
Zinc

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The common bean (*Phaseolus vulgaris* L.) is the third most important legume for human consumption worldwide (Broughton *et al*., 2003; Saltzman *et al*., 2013). Most of the protein consumed by poor populations comes from plant sources, which are rich in protein (Beebe, 2012), and beans play an important role in the human diet. Although they are much less important than cereals as a source of calories, beans often provide a significant percentage of carbohydrates. Like other legumes, they are also a key source of minerals, especially iron and zinc (Carvalho and Vasconcelos, 2013, FAO *et al*., 2015).

Bean is one of the most traditional crops of Colombian agriculture because it is part of the staple food of Colombian families; it is also a source of income for peasant families. It is important for food consumption due to its nutritional content of vegetable origin and an alternative for the rural population with scarce economic resources. Also, it is important to take into account what is recommended in the nutrition system of the World Health Organization (WHO) for the design of public policies, which considers the recommended daily allowance (Recommended Dietary Allowance, RDA) of such nutrients (Simpson *et al*., 2011; Tofiño *et al*., 2015). Likewise, it has been detected that beans have iron and zinc contents that can supply the daily requirements of these minerals (McClean *et al*., 2011).

Biofortification seeks to improve the nutrient density of primary food crops through conventional plant breeding, agronomic management, or genetic engineering (Blair *et al.,* 2008; Blair, 2013; Thavarajah *et al*., 2009). Currently, the carotenoids as sources of provitamin A, iron, and zinc are important due to the high prevalence of deficiencies of these micronutrients in children under five years of age and women on reproductive age in developing areas of Africa, Asia and Latin America (Saltzman *et al*., 2013; Nestel *et al.*, 2013). Therefore, increasing the concentration of bioavailable micronutrients (Ariza-Nieto *et al*., 2007) in edible crops (biofortification) has become a promising strategy in modern agriculture, which allows more nutritious foods to be accessed by more people and with the use of fewer resources (Nestel *et al*., 2006; Bouis and Welch, 2010; Blair *et al*., 2013; Vaz-Tostes *et al*., 2016).

As part of a biofortification program, new lines of Andean beans with high iron and zinc contents have been developed by the Andean plant breeding program of the Andean II, group led by Dr. Blair at CIAT (International Center for Tropical Agriculture). They are an alternative to solve the problem of public health in Colombia caused by a lack of micronutrients, which presents some degree of undernourishment, malnutrition, and anemia. These varieties of beans are developed to be grown in the Colombian Andean Region; they contain 60% more iron (82 ppm) and 50% more zinc (43 ppm), than the traditional varieties that have an average of 50 ppm and 28 ppm, respectively (Beebe, 2012; Brigide *et al*., 2014).

For this reason, the objective of this research was the evaluation of bush or climbing bean genotypes enriched with iron and zinc and adapted to production areas of mid-climate and altitudes (NUA or MAC lines) in farms of producers. Participatory evaluation methodologies were applied, and trials were carried out in three locations with seven and eight, bush and climbing beans respectively, using a local control variety (Uribe Rosado) and one resistant to anthracnose (G2333).

MATERIALS AND METHODS

Plant material

In three locations (Rionegro, Jardín, and Betulia), the NUA 30, NUA 35, NUA 45, NUA 56, CAL 96, and AFR 612 bush genotypes were planted, coming from the CIAT Agrosalud nursery and Dr. Blair's breeding program for Andean Nutrition (NUA). These genotypes were compared with the regional variety Uribe Rosado, which acts as a control. All NUA lines come from the backcrossing CAL 96×(CAL 96×G14519), according to Blair *et al.* (2010). AFR 612 was a genotype bred during the '90s for Africa, and CAL 96 is an improved bred Calima type for mid- climates.

The varieties of climbing beans planted were also from Dr. Blair's program for mid-climate and altitude adaptation (MAC): MAC 9, MAC13, MAC 27, MAC 31, MAC 33, MAC 52, MAC 54. The variety of G2333, accession of the germplasm bank of Mexican origin, was used as a control.

A randomized complete block design with four replications was used for each group of beans (bush and climbing separately). The planting distance for bushy beans was 0.20 m between plants and 0.80 m between rows in plots of 5 m in length, and for climbing beans was 0.20 m between plants and 1.0 m between rows. For each row, 25 seeds

were sown for a total of 100 plants per replication of each genotype. The climbing beans were supported with 2 m long bamboo canes tied together with polyethylene threads, while the bush beans were planted without any type of support.

Trials in Rionegro

For the experiment in the municipality of Rionegro the plantations were held in the Research Center/Experimental Station 'La Selva' with soils of Andisol type (TyPic Melanaquand/Medial Isothermal), a flatland with high contents of organic matter, high in calcium, potassium, phosphorus, and medium to low magnesium. Regarding the minor elements, medium to high contents was present (Table 1). According to the soil analysis, it is a land of medium to high fertility.

Trials in the municipality of Jardin

The farm's soil of a bean grower in this municipality has low organic matter content, very low in phosphorus, low in potassium, high in calcium and medium in magnesium; but very high in iron content and low in zinc; in general it is a soil of low fertility (Table 1).

Table 1. Soil chemical characteristics of the municipalities of Rionegro, Jardín and Betulia.

Site	pH	OM	Al	Ca	Mg	K	P	Fe	Cu	Mn	Zn	B
		%		$cmol_c\ kg^{-1}$					mg kg^{-1}			
Rionegro	5.4	20.2	-	8.1	1.58	1.83	80.4	229	6.7	6.5	14.3	0.20
Jardín	5.0	3.6	-	6.6	3.10	0.15	2.0	167	1.7	30	1.9	0.08
Betulia	4.8	5.8	2.4	5.6	2.30	0.09	2.0	201	5.5	160	2.9	nd

pH in water (1:1); OM: Organic Matter Walkley and Black; Al: KCl (1 M); Ca, Mg, K, and Na: Ammonium acetate 1 M; P: Bray II; S: Calcium Phosphate 0.008 M; Fe, Mn, Cu, and Zn: Olsen-EDTA; B: Hot water.

Trials in the municipality of Betulia

The soil studied in this municipality of Antioquia has average contents of organic matter, low in phosphorus, potassium, and medium in calcium and magnesium; very high in iron and manganese content and low in zinc; in general, it is a soil of low fertility (Table 1).

RESULTS AND DISCUSSION

Bush Bean Yields

In Table 2, it can be observed the production of the bred lines of biofortified bush beans. There were highly significant differences for the variables: pods per plant, beans per pod, and significant differences for the variables weight per row and total yield in kg ha^{-1}.

Location of Rionegro. The highest yield was obtained with the NUA 45 material, which was statistically equal to the Uribe rosado, CAL 96, and AFR 612 materials. The last two had the same performance statistically as the NUA 35 and NUA 30 materials. These materials exceeded the SCR3 genotype (1.45 t ha^{-1}) evaluated by Tofiño-Rivera *et al.* (2016); similarly, the genotype AFR 612 produced proper levels of yield and similar to those reported by Astudillo and Blair (2008). NUA 56 had a low yield, only reaching 1,390 kg ha^{-1} compared to 2,260 kg ha^{-1} of NUA 45 and approximately 2,000 kg ha^{-1} of AFR 612, CAL 96, and NUA 35. Regarding the number of pods per plant, none of the materials presented significant differences. For the variable number of grains per plant, the NUA 56 material was statistically superior to the others.

Location of Jardín. In this locality, the genotypes behaved similarly to those of Rionegro. In the lines of bush beans, there were significant differences in the three measured characteristics among the different materials evaluated. The highest yields were for the material NUA 45 and the control variety Uribe Rosado followed in order by the genotypes CAL 96 and AFR 612 (Table 2). These results are similar to those obtained by Tofiño *et al.* (2011) with biofortified materials for the Caribbean region. The performance of this location was low.

Regarding the number of pods per plant, the NUA 45 material was statistically different from the NUA 56. The others had a similar behavior among them. However, for the number of grains per pod, there were no significant differences between the materials.

Location of Betulia. There were significant differences between the different bush materials evaluated. The highest

yields were for the Uribe Rosado material and the AFR 612 material, followed in order by the CAL 96, NUA 45, and NUA 35 genotypes (Table 2); however, the performance is statistically similar among them but different from the NUA 56 and NUA 30 materials. During the evaluation, it occurred a period of intense rainfall, but the yields were medium. Maybe this environmental factor affected yields. For the number of grains per pod, the materials NUA 56 and NUA 30 were statistically lower than the others.

Table 2. Production of biofortified Bush bean lines in three locations of Antioquia.

Genotypes	Rionegro (C.I. La Selva)			Jardín (in farm)			Betulia (in farm)	
	Pods per plant	Grains per pod	Dry weight (kg ha^{-1})	Pods per plant	Grains per pod	Dry weight (kg ha^{-1})	Grains per pod	Dry weight (kg ha^{-1})
NUA 56	27 a	5.75 a	1,390 c	47 c	13 a	760.4 bc	5.38 c	796 c
NUA 30	25 a	4.00 c	1,719 bc	72 abc	18 a	739.6 bc	5.89 c	870 c
NUA 45	26 a	4.25 c	2,260 a	93 a	18 a	1,072.9 a	9.25 ab	1,370 ab
NUA 35	22 a	4.00 c	1,560 bc	87 ab	20 a	653.3 c	8.25 ab	1,222 ab
Uribe Rosado	30 a	5.00 b	1,967 ab	82 ab	18 a	1,052.1 a	1.20 a	1,778 a
CAL 96	27 a	4.00 c	2,046 ab	57 bc	19 a	968.8 abc	9.38 ab	1,396 ab
AFR 612	24 a	4.50 bc	2,060 ab	77 abc	15 a	885.4 abc	1.05 a	1,556 a
SD	1.88	0.31	258.08	12.78	1.88	135.69	2.77	275.43

Means with the same letter among the column do not differ statistically, Tukey's ($P \leq 0.05$).

The high yield of NUA 45 distinguishes in the studied area compared to other evaluations in Valle del Cauca (Colombia), where NUA 35 has been preferred for its precocity and high yields. Despite being an undetermined bush bean, NUA 56 has a low yield in both areas of Antioquia and Valle del Cauca.

Nutritive Quality of Biofortified Bush Beans

The overall mean for the locations was 58.6 mg kg^{-1} for iron concentration and 34.1 mg kg^{-1} for zinc concentration. With the ANOVA, significant differences were identified between the genotypes for both iron and zinc (Table 3). The mean comparison of each genotype showed that NUA 35 had a higher concentration of iron (71.05 mg kg^{-1}) and zinc (40.40 mg kg^{-1}), followed by CAL 96 with 60.0 mg kg^{-1} and 32.1 mg kg^{-1} of iron and zinc, respectively. The correlation between iron and zinc was highly significant, with a $P<0.0123$.

Table 3. Iron and zinc content for the seven genotypes evaluated in the locations of Jardin, Betulia, and Rionegro in the department of Antioquia.

Genotypes	Iron (mg kg^{-1})			Zinc (mg kg^{-1})		
	Jardín	Betulia	Rionegro	Jardín	Betulia	Rionegro
AFR612	57.59 a	52.57 b	57.06 b	31.10 a	31.86 ab	35.80 ab
CAL 96	49.96 a	51.36 b	60.06 b	25.60 a	26.15 c	32.05 bc
NUA35	55.91 a	66.77 a	71.05 a	27.72 a	33.60 a	40.40 a
NUA45	59.18 a	48.41 b	52.38 b	27.42 a	24.60 c	29.69 c
NUA56	59.40 a	54.10 b	59.40 b	26.93 a	29.73 abc	31.85 bc
Uribe Rosado	54.52 a	49.03 b	54.99 b	28.87 a	27.59 abc	34.77 bc
NUA30	-	-	55.11 b	-	-	34.28 c

Means with the same letter among the column do not differ statistically, Tukey's ($P \leq 0.05$).

It was determined that Rionegro had the highest mean among the three evaluated locations, and it was the place where the NUA 35 genotype obtained the highest concentration of iron and zinc. These results were similar to those reported by Tofiño *et al.* (2016) in genotypes for the Colombian Caribbean Region; it is necessary to clarify that the soils of the moderate and cold climate in Antioquia have high contents of Fe, different from the soils of the Caribbean Region, where previous studies were conducted with Mesoamerican biofortified beans but not Andean biofortified beans as studied in Antioquia. The bioavailability of some of the NUA lines is high (Ariza Nieto *et al.*, 2007).

Climbing Bean Yields

In Table 4, it is observed the production of the climbing beans. There were highly significant differences for the variables pods per plant, grains per pod and dry weight in kg ha^{-1} of grain.

Location of Rionegro. In this location, the material G2333 presented the highest number of grains per pod (8.25) and is statistically different from the others. The highest yields were for the climbing bean material G2333. This treatment was equal to the genotype MAC 27 and similar to the results of Blair *et al.* (2007). The other materials had similar performance. All these climbing genotypes had high yields (2 to 5 t ha^{-1}), agreeing with what was exposed by Sida-Arreola *et al.* (2015) in which biofortified crops must be of high yield and profitable for the farmer.

Location of Jardín. Concerning the number of grains per pod, the MAC 27 (4.56) and G2333 (5.31) presented similar behavior, being statistically superior to others. For the climbing beans, significant differences were found for the materials G2333 and MAC 27, which obtained high yields between 3.8 and 4.4 t ha^{-1} (Table 3). The other materials were equal in yield.

Location of Betulia. In the evaluation of the climbing beans, significant differences were observed between the treatments; yields fluctuated between 2,900 and 1,660 kg ha^{-1}. The highest production was for the material G2333, which was statistically different from the other materials.

Materials G2333 and MAC 27 obtained the highest production of grains per pod (2.95 and 2.41), respectively, being statistically different from the other materials. In this variable, the material with the lowest number of grains per pod (1.25) was MAC 52. For the dry weight, the same trend was observed, being statistically the best material the G2333 material with 3,933 kg ha^{-1} and that of lower response MAC 52 with 1,660 kg ha^{-1}.

Table 4. Production of climbing bean lines adapted to mid-climate and altitude (MAC) in three locations of Antioquia.

Genotypes	Rionegro (La Selva)		Jardín		Betulia	
	Grains per pod	Dry weight (kg ha^{-1})	Grains per pod	Dry weight (kg ha^{-1})	Grains per pod	Dry weight (kg ha^{-1})
MAC 31	6.00 bc	3,142 b	2.250 b	1,875 b	1.575 cd	2,100 cd
MAC 9	4.50 e	3,227 ab	3.125 b	2,064 b	1.588 cd	2,117 cd
MAC 52	5.00 de	3,092 b	2.562 b	2,135 b	1.250 f	1,660 f
MAC 33	5.00 de	3,127 b	2.812 b	2,343 b	1.650 cd	2,200 cd
MAC 27	5.00 de	4,056 a	4.562 a	3,802 a	2.413 b	3,217 b
G2333	8.25 a	4,073 a	5.312 a	4,427 a	2.950 a	3,933 a
MAC 13	6.00 bc	3,121 b	3.312 b	2,760 b	1.913 c	2,550 c
MAC 54	5.50 cd	3,054 b	3.062 b	2,552 b	1.814 cd	2,400 cd
MAC 4	6.25 b	2,875 b	3.062 b	2,552 b	1.950 c	2,600 c
SD	0.80	336.47	0.71	626.44	0.36	483.75

* Means with the same letter among the columns do not differ statistically, Tukey's ($P \leq 0.05$).

CONCLUSIONS

For biofortified bush beans, the highest yields were for NUA 45, surpassing CAL 96 and AFR 612, two non-biofortified beans. The yields of these were similar to the local control variety Uribe Rosado in Jardín but not in Betulia, Antioquia. For climbing beans, the highest yields in all locations of the municipalities of Rionegro, Jardín, and Betulia were for the climbing bean material G2333. This material is equal to the MAC 27 genotype. The genotype that had the lowest yields was the material MAC 52. While the majority of MAC lines were of spotted red color, MAC 27 has a full red color that could compete with the high-priced varieties in category "Bola Roja," although they are more elongated in the form of a seed. The materials NUA 45 and MAC 27 are shown as promising beans and potential varieties for their high yields.

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Research article

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Leaves per tiller as the criterion to determine optimum defoliation frequency in pastures of *Brachiaria decumbens*

Hojas por macollo como criterio para determinar la frecuencia optima de defoliación en pasturas de *Brachiaria decumbens*

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ABSTRACT

Two studies were performed to establish the best defoliation frequency in *Brachiaria decumbens* using the number of leaves per tiller as a criterion. In the first study, 3,500 tillers with 2, 3, 4, 5, or 6 leaves were collected in 10 farms. The number of green, senescent, and dead leaves was counted. A pooled sample of tillers by each farm with a given number of leaves was analyzed for nutritional quality; a complete randomized block design was used for this study. In a second study, it was implemented a complete ramdomized design with repeated measures over time (2 defoliations), and the effect of defoliating *B. decumbens* at 3, 4, or 5 leaves per tiller on its growth, dry matter yield, and nutritional quality were evaluated. In the first study, all leaves remained green until the tiller had 3 leaves. At the fourth leaf, few tillers (2.3%) had senescent leaves, but at 6 leaves, most tillers had senescent (40.6%) or dead (24.4%) leaves. Crude protein and digestibility decreased as the number of leaves in a tiller increased (P<0.001). In the second study, the rate of dry matter accumulation was the highest for plots defoliated at 4 leaves (P<0.05). Green stems (43.9%) represented a larger proportion of dry matter than green leaves (31.4%), and their proportions were similar among treatments. Crude protein and digestibility of stems decreased as the number of leaves per tiller increased (P<0.001), but the quality of leaves was similar. These findings suggest that these pastures should be defoliated at 4 leaves per tiller.

Keywords:
Brachiaria grass
Grazing management
Signal grass
Tropical grasses
Urochloa decumbens

RESUMEN

Se realizaron dos estudios para establecer el punto óptimo de defoliación del *Brachiaria decumbens* usando como criterio el número de hojas por macollo. En el primer estudio, se cosecharon 3500 macollos de 2, 3, 4, 5, o 6 hojas en 10 fincas. Se contó el número de hojas verdes, senescentes o muertas. Se determinó la calidad nutricional de los macollos, para este primer estudio se utilizó un diseño de bloques completos al azar. El segundo estudio se utilizó un diseño experimental completamente aleatorizado con medidas repetidas en el tiempo (2 defoliaciones), se evaluó el efecto de defoliar *B. decumbens* a 3, 4, o 5 hojas por macollo, sobre el crecimiento, rendimiento de la materia seca y la calidad nutricional. En el primer estudio, las hojas permanecieron verdes hasta la tercera hoja. A partir de la cuarta hoja, se presentaron algunos macollos con hojas senescentes (2,3%) pero a las 6 hojas la mayoría estaban senescentes (40,6%) o muertas (24,4%). La proteína y la digestibilidad disminuyeron al aumentar el número de hojas por macollo (P<0,001). En el segundo estudio, la tasa de acumulación de materia seca fue mayor para las pasturas defoliadas con 4 hojas. Los tallos verdes (43,9%) representaron una mayor proporción de la materia seca que las hojas verdes (31,4%). La proteína y la digestibilidad de los tallos disminuyó con el número de hojas (P<0,001) mientras que la calidad de las hojas fue similar. Este estudio sugiere que estas pasturas deben cosecharse a 4 hojas por macollo.

Palabras clave:
Brachiaria amarga
Manejo del pastoreo
Pastos tropicales
Urochloa decumbens

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Livestock production is the main economic activity of the Colombian piedmont eastern plains (Vergara, 2010), which is based mainly on pastures of *Brachiaria* spp. In the Araucano piedmont, one of the introduced grasses of greater adoption is *Brachiaria decumbens* Basilisk (synonymous, *Urochloa decumbens* Stapf R. D. Webster) for its adaptation to acid soils and response to fertilization. This pasture is managed in rotational grazing without using specific criteria to determine its optimum defoliation frequency (Rincón *et al*., 2008).

In the last two decades, considering pasture growth dynamics, significant progress has been made adjusting the management of pastures regarding the optimum defoliation frequency. For *Lolium perenne*, the use of leaves per tiller has been widely studied and adopted by farmers (Fulkerson and Slack, 1994; Fulkerson and Donaghy, 2001; Solomon *et al*., 2017). This criterion was developed by the observation that, in this specie, tillers maintain a maximum of three green leaves, and senescence begins in the leaf closer to the ground when the fourth leaf emerges (Fulkerson and Slack, 1994). Therefore, *L. perenne* pastures should be harvested at this point to avoid the accumulation of dead material and maintain pasture quality.

The number of leaves per tiller as a criterion to determine defoliation frequency may be a better parameter to establish defoliation time than others such as days to defoliation, height to defoliation, or light interception. Days to defoliation may vary due to environmental conditions (temperature, light, rain), and a given number of days will be valid only under similar environmental conditions. Herrero *et al.* (2.000a, 2000b) suggested that pasture management should consider environmental conditions to determine defoliation frequency and that the number of leaves per tiller could be an applicable pasture management guideline since leaf appearance and pasture growth relates to environmental conditions. Pasture height (Gouveia *et al*., 2017) is a parameter that does not consider pasture quality that has a profound effect on animal performance. Finally, to defoliate pastures at 95% light interception has shown promising results (Pedreira *et al*., 2017a) but requires special apparatus to be measured that limits the use of this technology in practical conditions.

In tropical pastures, Gomide *et al.* (2007) suggested that for *Panicum maximum*, the number of leaves per tiller can be used to establish the resting period of a pasture. These authors found that this specie harvested with fewer leaves per tiller (2.5) was more productive than those harvested with a larger number of leaves per tiller (4.5) due to more frequent defoliation that stimulates pasture growth. In *B. decumbens*, the number of green leaves that a tiller can maintain varies according to management and season. Fagundes *et al.* (2006) observed that tillers of *B. decumbens* fertilized with nitrogen maintained 5.1 green leaves per tiller while unfertilized maintained 6.7. Similarly, Da Silva *et al.* (2012) found that unfertilized *B. decumbens* keep up to 6.7 green leaves while fertilized 5.2. Santos *et al.* (2011) observed that the number of green leaves in a tiller was lower in winter (2.9) than in summer (5.4). Santos *et al.* (2011) observed that this grass managed under continuous grazing and defoliated to maintain a constant (25 cm) or variable 15- to 25-cm height, tillers keep close to five green leaves during spring and summer but only 2.9 during winter. Therefore, environmental conditions may alter the number of green leaves that a tiller supports, and research should be performed under particular conditions to establish appropriate harvesting time for this species using this criterion.

There is no information to infer the appropriate number of leaves per tiller in *B. decumbens* to avoid senescence of leaves in Araucano piedmont; therefore, the objective of this study is to generate defoliation parameters for *B. decumbens*, considering the number of green leaves that a tiller can maintain.

MATERIALS AND METHODS

Two studies were conducted to determine the optimum defoliation frequency of *B. decumbens* pasture using the number of green leaves per tiller as a criterion.

Study 1

Ten cattle farms were selected from the Araucano piedmont (municipality of Tame in the department of Arauca) to determine the moment when the first leaf that emerged after grazing begins its senescence. All the farms have *B. decumbens* cv. Basilisk pastures. Climatic conditions were similar among farms. The average temperature was 25.3 °C, a dry season of

four months (December to March), a rainy season from April to November with total accumulated rainfall of 2,047 mm in 2015 (Figure 1). Soils of the region are strongly acid (<4.5), with a high aluminum saturation (2.4 meq $100g^{-1}$), low cation exchange capacity (<9 meq $100g^{-1}$), low content of organic carbon (<1.3 %), and P (<20 mg kg^{-1}) (IGAC, 2017). Most farms (80%) did not use fertilization; pastures were rotationally grazed and rainfed. Stocking rates vary among farms due to differences in the primary productivity of pastures. From September 3 to October 10, 2015, 350 tillers were collected in each farm; seventy tillers per each maturity stage (2, 3, 4, 5, or 6 leaves per tiller).

The number of leaves per tiller was established, counting the number of leaves from the bottom of the tiller to its top. Data were recorded considering expanding, expanded, senescent, and dead leaves: a) Expanding leaf: the blade of the leaf was not fully exposed, b) expanded leaf: the ligule was visible, c) senescent: less than 50% of the leaf blade showed signs of senescence. d) Dead: more than 50% of the leaf blade showed signs of senescence.

A pooled sample by farm of leaves per tiller (2, 3, 4, 5, or 6), were dried at 65 °C for 48 hours and ground in a Wiley type blade mill to determine de nutritional quality of the tiller with a different number of leaves. Samples were analyzed for dry matter (DM), ash, crude protein (CP) (AOAC, 2012), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest *et al.*, 1991) and *in vitro* digestibility of dry matter (IVDDM) (Tilley and Terry, 1963).

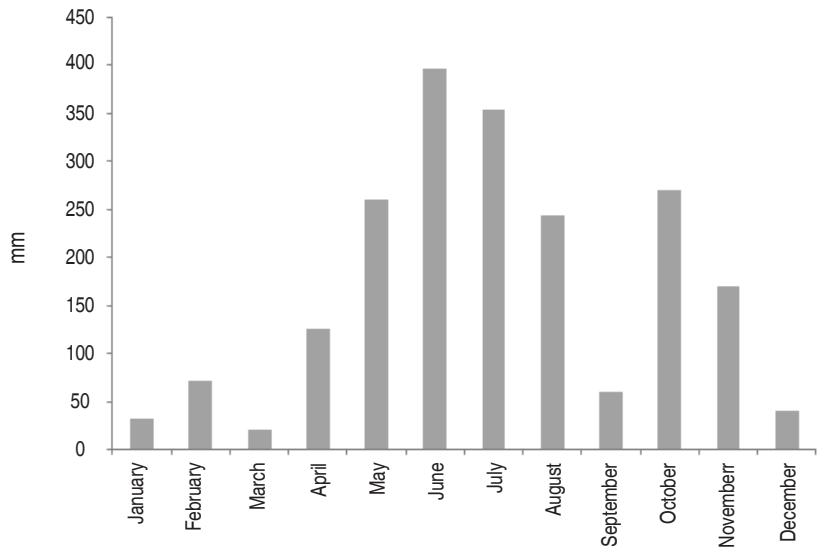

Figure 1. Rainfall (mm) in the Araucano piedmont during 2015 (Based on historical data collected in the region, IDEAM, 2020)

Study 2

This experiment was conducted in a farm located in the municipality of Tame (6°52'36.55" N, 71°68'96.23" W), in an established pasture of *B. decumbens* cv. Basilisk. The study began on September 15 and ended on December 15, 2016. Rainfall was 256 mm during the experimental period, and the average temperature was 26.7 °C. However, the rain was not evenly distributed and did not rain in the last 20 days of the experiment. According to soil composition (Table 1), dolomite lime (300 kg ha^{-1}), diammonium phosphate (250 kg ha^{-1}), urea (11 kg ha^{-1}), and sulfur (18 kg ha^{-1}) were applied in an area of 250 m^2. The selected pasture area was defoliated before fertilizing at 25 cm height on September 27, 2016.

The treatments consisted of three defoliation frequencies of the pasture, taking as a criterion the number of leaves per tiller (3, 4, or 5 leaves). Nine plots of 5×4 m were established as experimental units assigning one of the treatments randomly to each plot (3 repetitions per treatment). The

methodology proposed by Fulkerson *et al.* (2010) was used to determine the number of leaves per tiller. Every 2 to 3 days, 15 tillers were counted in each plot, considering the time of harvest when at least 70% of them had the number of leaves of the treatment. The plots were defoliated in two consecutive periods at 25 cm height, as recommended by Rincon *et al.* (2008). The two periods for each treatment were evaluated without interruption.

Table 1. The soil composition in study 2.

pH	OC* (g kg)$^{-1}$	TN* g kg^{-1}	Interchangeable bases cmol(+) kg^{-1}				CEC* meq 100g^{-1}	P** mg kg^{-1}	Microelements mg kg^{-1}				Texture
			Na	K	Ca	Mg			Cu	Fe	Zn	Mn	
5.4	5.5	2.2	<0.10	0.44	4.27	0.68	3.87	16.8	0.43	74.36	2.64	7.56	SL*

* OC-Organic carbon, TN-Total nitrogen, CEC – Cation exchange capacity, SL-Sandy loam texture, **P- Available phosphorus by Bray II method.

Morphogenic characteristics were measured by selecting 15 tillers in each plot. These tillers were tagged and followed throughout the experiment every 2 or 3 days. In each of the 15 tillers, data were recorded regarding a) Days of the appearance of leaves, b) length of each leaf from its appearance until ligule exposition, c) alive and dead leaves. According to Curcelli (2009), recorded data were used to estimate leaf appearance rate (LAR), phyllochron, leaf elongation rate (LER), the final length of the leaf (mean length of all leaves in a tiller), and the number of dead leaves (DL)

Pasture undisturbed height was estimated by placing a transparent sheet on top of the pasture and measuring with a ruler the distance from the soil to the sheet. Ten observations per plot were taken every 7–8 days until harvest.

Pasture growth and dry matter yield were determined by harvesting in each plot an area of 0.25 m^2 every 7 to 8 days at a 25 cm height. A sample of the cut pasture of each plot was dried in an oven (60 °C for 48 hours) to estimate dry matter and nutritional quality by wet chemistry as described above (Study 1). Dry matter and nutrient yield were estimated considering the area of the plot and the nutrient composition of the pasture.

Pasture components were measured at each defoliation frequency by harvesting a pasture area (25×25 cm^2) at ground level in two points of each plot. The harvested biomass was separated into three components: green leaves, green sheaths and stems, and dead material. Then, dried in an oven (60 °C for 48 hours) to estimate dry matter and nutritional quality (CP, NDF, ADF, and ash) by near-infrared reflectance spectroscopy (NIRS), employing a DS2500 FOSS equipment calibrated against wet chemistry (Ariza-Nieto *et al.*, 2017). The IVDDM was determined by the Tilley and Terry (1963) method.

Statistical analysis

Study 1

A complete randomized block design was applied using the GLM procedure of SAS®. Sources of variance were the number of leaves per tiller (2, 3 4, 5, 6), farms (Blocks), and the experimental error. Statistical assumptions for linear models were achieved. An analysis of Variance (ANOVA) was performed at a significant level of 5% (P<0.05), and multiple comparisons among means were performed using the Tukey test (Martinez *et al.*, 2011a).

Study 2

A complete randomized design was used. Dry matter and nutrient yields, nutritional quality, morphogenic characteristics, and structural components were analyzed using the GLM procedure of SAS®, performing an analysis of variance with the level of significance of 5% (P<0.05). Source of variance were the number of leaves per tiller (3, 4, 5), defoliation (1, 2), and experimental error. Tukey test was used to determine differences between mean treatments (Martinez *et al.*, 2011a).

Dry matter accumulation rate (kgDM ha^{-1} d^{-1}) of each treatment (leaves per tiller) was determined by simple

linear regression (Growth days vs. DM yield) and slopes of each treatment compared using the student T-test. The assumptions of the models were homogeneous experimental material and the experimental error as an independent random variable with normal distribution, mean 0, and common variance. Shapiro-Wilk test was computed to determine the normality (Martinez *et al.*, 2011b).

RESULTS AND DISCUSSION

Study 1

In the first study, the evaluation of a large number of tillers (3,500) in ten cattle farms suggests a similar behavior of leaves senescence in a tiller in the pastures of *B. decumbens* regardless of management and environmental conditions among farms (Figure 2). It also suggests that the number of leaves per tiller is a valid parameter to give recommendations of the optimum defoliation frequency for the Araucano piedmont region. In each tiller, the senescence started in the leaf that appeared first (leaf closest to the ground), and leaves of a tiller remained green until the third leaf. From leaf 4, some senescent leaves were seen in the tillers, although their proportion was low (2.3%). From the fifth leaf, dead leaves appear, but also in a low proportion of tillers (3.3%). From leaf 6, most of the tillers had senescent or dead leaves. Therefore, data suggested that the senescence of the first leaf began in most tillers when these reached the fifth leaf since, at a stage of six leaves, most tillers had at least one senescent or dead leaf (Figure 2). Therefore, defoliate *B. decumbens* at 6 leaves per tiller would seem inappropriate. At this point, the additional leaf would imply the senescence of the first leaf, which results in a constant number of green leaves (5) in a tiller and a decrease in the nutritional quality of the pasture. From leaf 6, any additional leaf will result in an accumulation of senescent and dead leaves from there onwards.

Additionally, the presence of dead leaves in the pasture would be undesirable since bovines prefer green leaves over dead leaves (Rattray *et al.*, 2007), which are located in the lower layer of the canopy, which difficult their intake. Additionally, these leaves have a lower nutritional quality than green leaves (Gomide *et al.*, 2001). Defoliating pastures with a greater number of leaves per tiller (>5 leaves) would have other disadvantages. Research on tropical pastures has found that increasing the rest period (number of leaves per tiller) in a pasture results in pastures with greater height (Maranhão *et al.*, 2010) which causes elongation of stems due to the elevation of the apical meristem in search of light and increases the shade of leaves near to the ground inducing their senescence (Lara and Pedreira, 2014).

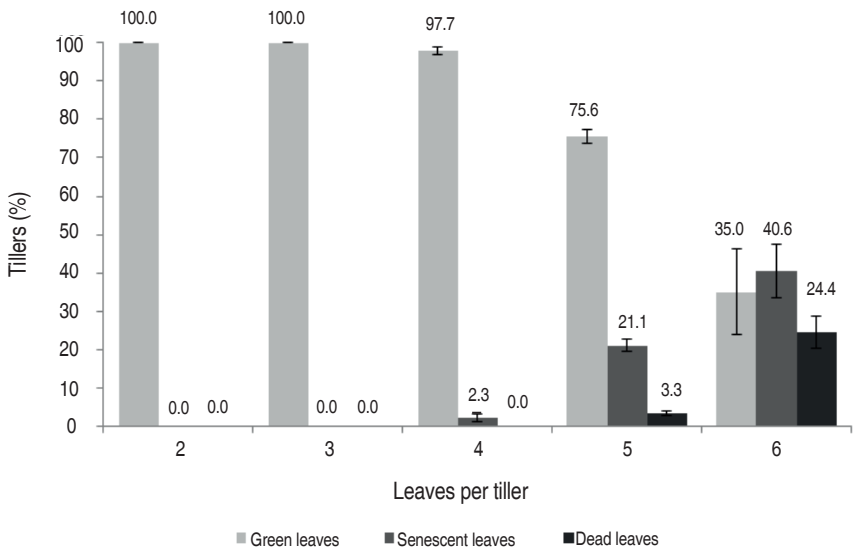

Figure 2. Proportion of tillers with green, senescent, and dead leaves in 10 farms of the Araucano piedmont. Error bars represent the standard deviation (SD) of the mean.

It may be advisable to defoliate the pasture with fewer leaves per tiller (<5), considering the nutritional quality. In this study, maturity measured as the number of leaves per tiller was related to a linear manner with most of the nutritional parameters of the tillers. In particular, the variations in IVDDM (r^2=0.40) and ADF (r^2=0.55) were more closely associated with the change in the number of leaves per tiller.

In vitro digestibility of dry matter (IVDDM) of tillers decreased with the increase in the number of leaves per tiller, being similar between tillers with 2 or 3 leaves and tillers with 4, 5, or 6 leaves. Crude protein decreased 2.9% from 2 to 6 leaves per tiller. NDF and ADF increased with the number of leaves per tiller but were similar among tillers with 2 or 3 leaves and 4, 5, or 6 leaves (Table 2).

Table 2. Nutritional quality of *Brachiaria decumbens* tillers defoliated with 2, 3, 4, 5, or 6 leaves in pastures of ten cattle farms of the Araucano piedmont.

	Leaves per tiller						
	2	3	4	5	6	SEM	Pr>F
Samples (n)*	10	10	10	10	10		
IVDDM (%)	72.8 a	71.4 ab	69.6 bc	68.3 c	67.1 c	2.02	<.0001
CP (%)	11.6 a	11.2 ab	10.3 abc	9.54 bc	8.7 c	1.51	0.0012
NDF (%)	59.8 a	61.2 ab	62.3 bc	63.3 c	63.6 c	1.46	<.0001
ADF (%)	26.3 d	27.3 cd	28.8 bc	30.1 ab	30.4 a	1.22	<.0001
Ash (%)	6.8	7.1	7.4	7.2	7.2	0.49	0.07

* Pooled samples of tillers with 2, 3, 4, 5 or 6 leaver by farm
Different letters in a row indicate statistical differences
IVDDM – *In vitro* digestibility of dry matter, CP – Crude protein, NDF – Neutral detergent fiber, ADF – acid detergent fiber

The changes in nutritional quality were slow, and quality was similar between two consecutive leaves (Table 2). A larger proportion of senescent and dead leaves in the tillers (tiller with 6 leaves) did not accelerate the loss of nutritional quality, probably due to their relatively low proportion compared to the total green leaves in the tillers (Figure 3).

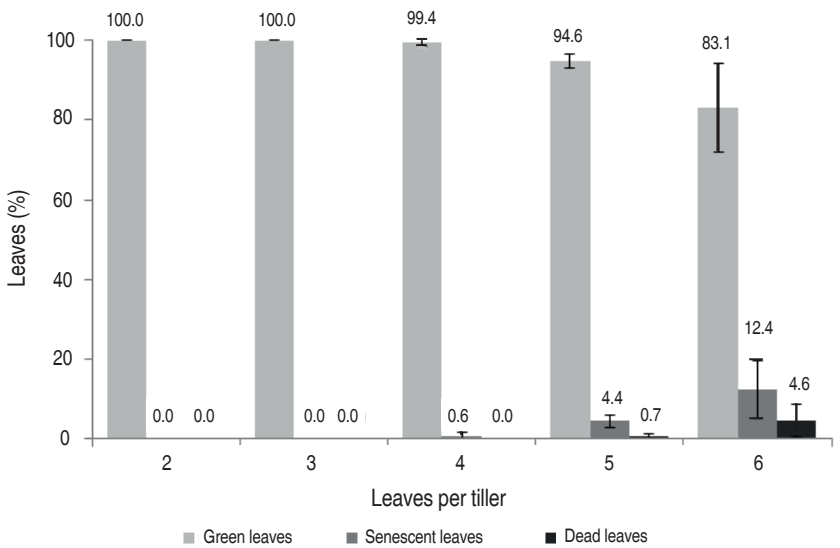

Figure 3. Percentage of green, senescent, and dead leaves from total leaves in tillers of *B. decumbens* in 10 cattle farms of the Araucano piedmont. Error bars represent the standard deviation (SD) of the mean.

The effect of increasing leaves per tiller on the reduction of the nutritional quality of the pastures has been documented in both temperates (Solomon *et al.*, 2017) and tropical grasses (Agnusdei *et al.*, 2011). In *Chlorys guayana*, Agnusdei *et al.* (2011) observed that increasing the number of leaves in a tiller and leaf stage reduced the *in vitro* digestibility of dry matter that was explained mainly by a reduction in the digestibility of leaf neutral detergent fiber.

In the present study, the highest nutritional quality was observed when tillers had two leaves. Defoliating the plants at two leaves per tiller increases defoliating frequency, and some authors point out that increasing defoliation frequency could compromise pasture recovery and that pastures may be depleted over time (Chilibroste *et al.*, 2000). However, studies with tropical pastures suggest that higher defoliation frequencies (Rizato *et al.*, 2019), or a lower pasture height (Afonso *et al.*, 2018) generate a higher pasture quality without sacrificing production. Consequently, higher yields of meat or milk per hectare at the higher nutritional quality of the pasture may be expected (Congio *et al.*, 2018). Therefore, optimum defoliation frequency for these pastures may be achieved with a lower number of leaves per tiller (higher quality) as long as the pasture nutrients yield is not compromised.

Study 2

In study 2, each additional leaf in a tiller required 8 to 9 days to appear. Therefore, the treatment of 3 leaves per tiller requires 16 to 18 days less than 5 leaves to be ready for defoliation (Table 3). Although pasture DM yield increased as the number of leaves in tillers increased from 3 to 5 leaves, results suggested that pasture may be defoliated at 4 or 5 leaves per tiller without sacrificing DM yield. Similar to that observed in the first study, IVDDM and CP decreased as the number of leaves per tiller increased, but the magnitude of these changes depends on defoliation (interaction). Fiber components (NDF, ADF, and lignin) increased as the number of leaves increased, but changes per each additional leaf were small (1 to 2%) (Table 3). Therefore, defoliating pastures of *B. decumbens* with 4 leaves per tiller resulted in an increase of the yield of digestible nutrients and CP as compared with 3 leaves per tiller and had the largest yield of NDF and ADF (Table 3). The pastures defoliated at 4 leaves per tiller had the largest DM accumulation rate (143.5 kgDM ha^{-1} d^{-1}) while those harvested at 3 (114.4 kgDM ha^{-1} d^{-1}) or 5 (92.6 kgDM ha^{-1} d^{-1}) leaves per tiller were similar to each other (P<0.05). Fernández *et al.* (2001) reported that growth and accumulation rates might be reduced with the increase of age in tropical pastures. As pastures mature, the net rate of photosynthesis is reduced (Lopes *et al.*, 2013), probably due to shading that occurs when the number of leaves per plant increases, reducing the assimilation of CO_2 (Hernández *et al.*, 2000).

The plant adapts to lower penetration of light, generating an increase in the length of stems by the action of the phytochrome, providing a greater height in the pasture. This method of avoidance of shading occurs by raising the apical meristem of tillers that seeks light to ensure their production and survival (Smith, 1982). This would explain the lower performance of pastures harvested at 5 leaves, but not the lower performance of pastures harvested at 3 leaves when compared with pastures harvested at 4 leaves per tiller. However, the observations of the morphogenic characteristics would seem to favor the treatment of 3 leaves per tiller that in the second defoliation had a shorter phyllochrone, a faster leaf appearance rate, and a shorter height. This treatment also had a faster leaf elongation in the first defoliation (Table 4).

Skinner and Nelson (1995) suggested that when the size of the tiller is short (less height), the leaves make a shorter route for its emergence at the end of the sheath. In the second study, the height was linearly and negatively related to the leaf appearance rate and to the phyllochron, which suggests that the longer the stem, the greater the length of the sheath, the appearance of new leaves becomes slower. Menezes *et al.* (2019) reported that the higher defoliation frequency increases the leaf expansion rate in *Brachiaria brizantha*; therefore, the tiller with fewer leaves has a higher leaf expansion rate. This behavior can be attributed to a greater renewal of tissues. More frequent defoliations facilitate a greater elimination of tissues and allow greater production of leaves, possibly by better penetration of light (Marcelino *et al.*, 2006). Such behavior was observed in the first defoliation where the faster leaf elongation rate was observed in tillers with a lower number of leaves, but not in the second one, where no differences among treatments were observed. However, in the second defoliation, leaf appearance was larger for tiller with fewer leaves (Table 4).

Table 3. Days to harvest, dry matter yield, nutritional quality, and nutrient yield of pastures of *Brachiaria decumbens* defoliated at 3, 4, or 5 leaves per tiller (LT) in two consecutive defoliations (DF).

Variable	DF 1			DF 2			Main effects					*P*			
	LT			LT			DF		LT						
	3	4	5	3	4	5	1	2	3	4	5	SEM	LT×DF	DF	LT
Days to defolation	19	27	35	22	32	40	27	31.3	20.5	29.5	37.5				
Yield (t DM ha^{-1} per DF.)	2.08	3.66	3.52	2.21	5.40	3.52	3.11	3.71	2.14 a	4.53 b	3.55 ab	0.81	0.185	0.167	0.023
Nutritional quality (%DM)															
IVDDM	64.6 a	61.3 a	52.3 b	60.3 a	50.5 b	51.4 b	59.4	54.1	62.5	55.9	51.8	1.3	0.002	0.0002	0.0002
CP	15.1 a	14.9 a	11.7 b	13.7 a	10.0 bc	7.7 c	13.9	10.2	14.4	12.4	9.4	0.9	0.035	0.0001	0.0001
NDF	59.4	62.0	65.5	65.4	67.9	66.6	62.3 a	66.6 b	62.4 a	64.9 ab	66.1 b	2.4	0.225	0.0104	0.0196
ADF	30.3	29.3	31.8	31.1	33.3	33.0	30.5 a	32.5 b	30.7 a	31.3 ab	32.4 b	1.1	0.086	0.0087	0.0156
Ash	6.5 a	6.7 ab	7.7 b	7.7	7.3	7.6	7.0	7.5	7.1	7.0	7.6	0.3	0.028	0.0111	0.3846
LG	1.4	1.8	2.0	1.7	1.7	1.8	1.7 a	1.7 a	1.6 a	1.7 ab	1.9 b	0.2	0.448	0.9639	0.0178
Nutrient yield (t ha^{-1} per defoliation)															
Digestible DM	1.35	2.25	1.87	1.34	2.73	1.81	1.83	1.96	1.34 a	2.49 b	1.84 ab	438	0.535	0.537	0.032
CP	0.32	0.55	0.43	0.31	0.54	0.27	0.43	0.37	0.31 a	0.54 b	0.35 ab	88	0.345	0.213	0.030
NDF	1.24	2.28	2.35	1.45	3.68	2.35	1.95	2.49	1.34 a	2.97 b	2.35 ab	559	0.142	0.088	0.020
ADF	0.63	1.07	1.13	0.69	1.81	1.17	0.95	1.22	0.66 a	1.44 b	1.15 ab	275	0.116	0.078	0.019

Different letters within a row indicate statistical difference.
DM- Dry matter, IVDDM – *In vitro* Digestibility of Dry Matter, CP – Crude protein, NDF – Neutral detergent fiber, ADF – acid detergent fiber, LG- Lignin

Table 4. Pasture height, leaf growth characteristics, and structure of *Brachiaria decumbens* pastures defoliated at 3, 4, or 5 leaves per tiller (LT) in two consecutive defoliations (DF).

Variable	DF1 LT			DF2 LT			Main effects DF		Main effects LT			P			
	3	4	5	3	4	5	1	2	3	4	5	SEM	LT×DF	DF	LT
Pasture height (cm)	50.2	48.2	51.4	55.0 a	74.5 b	73.1 b	49.9	67.5	52.6	61.3	62.2	1.82	0.0001	<0.0001	0.01
Leaf															
Elongation (cm leaf^{-1} d^{-1})	0.93 a	0.58 b	0.40 b	0.41	0.35	0.31	0.63	0.36	0.67	0.46	0.35	0.06	0.002	<0.0001	0.0008
Appearance (leaf tiller^{-1} d^{-1})	0.14	0.14	0.13	0.17 a	0.13 b	0.11 b	0.14	0.14	0.16	0.14	0.12	0.01	0.002	0.5000	0.0083
Phyllochrone (d leaf^{-1})	6.7	6.8	7.3	5.7 a	7.4 b	8.8 c	6.9	7.3	6.2	7.10	8.0	0.35	0.002	0.0700	0.0073
Senescence (dead leaves per tiller)	0.00	0.09	0.22	0.32	0.76	0.98	0.10 b	0.69 a	0.16 b	0.42 ab	0.60 a	0.17	0.150	0.0004	0.0500
Final length (cm)[1]	17.7	15.6	13.8	9.14	11.2	12.5	15.7 a	10.9 b	13.4	13.4	13.2	2.05	0.050	0.0020	0.9700
Pasture structure															
Green leaves (%)	38.3	31.7	32.6	27.2	28.6	30.2	34.2	28.6	32.7	30.1	31.4	5.23	0.33	0.060	0.77
Green stems (%)	44.1	37.6	38.2	44.8	51.9	46.8	39.9 b	47.8 a	44.4	44.8	42.5	3.73	0.05	0.004	0.72
Dead leaves (%)	11.2	13.5	15.0	15.0	8.5	10.1	13.2	11.2	13.1	11.0	12.6	2.49	0.03	0.130	0.59
Dead stems (%)	6.4	17.2	14.2	13.0	11.0	12.9	12.6	12.3	9.7	14.1	13.5	4.14	0.09	0.880	0.44

Different letters in the same row indicate statistical differences.
[1]Length mean of all leaves of a tiller.

Some literature favors a lower number of leaves per tiller as the criterion to defoliate the pasture. Martuscello *et al.* (2017) mention in their research that light determines leaf appearance rate, the tillering, and the leaf expansion rate. As the leaf area index increases, the uptake of light radiation by the plants' decreases, resulting in delayed emergence of tillers and leaves and therefore decrease in the photosynthetic rate (Brown, 1984). However, a higher frequency may have limits since high frequencies may deplete storage carbohydrates. Fulkerson and Donaghy (2001) showed that in ryegrass requires at least two leaves per tiller to have an adequate subsequent regrowth. On the other hand, Pedreira *et al.* (2017b) reported that more frequent defoliation (base in a 95% light interception) accumulates more underground carbohydrates than less frequent defoliation in *B. brizantha*.

In the present study, the final length of leaves did not change due to defoliation treatment (Table 4). In tropical grasses, Da Silva *et al.* (2012) associated the final length of the leaves with nitrogen fertilization and Pontes *et al.* (2003) with residual height, aspects that were uniform among treatments. However, differences in final length were found between defoliation periods where the first defoliation presented longer leaves (Table 4), suggesting that changes in environmental conditions may affect leaf growth. Santos *et al.* (2011) observed that during winter, leaves were shorter. In the present study, temperatures were slightly lower for the second defoliation (0.5 to 1.0 ˚C) that may explain shorter leaves for the second defoliation.

The proportion of components (leaves, stems, dead material) of the pasture did not change due to defoliation treatment (number of leaves per tiller). Green leaves and green stems represented close to three-quarters of dry matter while dead material (leaves and stems) one fourth. Green stems were the largest proportion of dry matter (Table 4). Gouveua *et al.* (2017) suggested that more frequent defoliation reduces the proportion of stems and dead material. In the present study, the proportion of stems and dead material was similar among treatments. Since avoiding the senescence of leaves was one of the criteria used in the selection of defoliation treatments (number of leaves per tiller), it could be expected that the proportion of dead material would be similar among defoliation treatments. This result would indicate that within the range studied (3 to 5 leaves per tiller), there would be no significant differences in the proportion of pasture components, which in practical terms would imply that the pasture could be managed among these ranges of the number of leaves per tiller without changing these parameters.

In the second study, defoliating the pasture of *B. decumbens* at a lower number of leaves per tiller improved its nutritional quality, similar to the results observed in the first study. Crude protein was increased in green leaves, green stems, and dead stems as the number of leaves decreased at defoliation. IVDDM increased as the number of leaves decreased in the tiller for the stems but varied among defoliations for green leaves. The concentration of NDF in the leaves was similar among treatments but increased in the green stems, and dead leaves as the number of leaves increased in a tiller (Table 5). Pedreira *et al.* (2017a) observed that more frequent defoliation resulted in a higher CP and *in vitro* organic matter digestibility in this grass in most conditions. However, stubble height and season may modulate the response of the pasture. Merlo-Maydana *et al.* (2017) also reported a positive effect of harvesting *Brachiaria* pastures at younger ages on the nutritional quality. In the present study, IVDDM decreased more rapidly in stems than in leaves because of defoliating the pasture with a greater number of leaves per tiller. Stems had an acceptable digestibility (61.4%) when harvested at earlier stages, while leaves had an IVDDM almost constant (Table 5).

The results of the present study suggest that harvesting pastures with fewer leaves per tiller would improve nutritional quality in both leaves and stems. Although cattle are highly selective and prefer leaves over stems (Santos *et al.*, 2013), harvesting pastures at a younger age with stems of better quality could have significant impacts on pasture digestibility and intake, as these represented an important part of the plant in *B. decumbens*. Additionally, the results of leaf expansion and appearance rate would also favor harvesting the pasture at a lower number of leaves per tiller. However, the best performance in DM accumulation rates and nutrient yield was observed when pastures were defoliated at 4 leaves per tiller. The interactions observed in some variables between the first and the second defoliation, would suggest that it is recommended to repeat experiments of this nature with longer experimental periods where the structural changes in the plant caused by defoliation frequency are consolidated.

Table 5. Nutritional quality of green leaves, green stems, dead leaves, and dead stems of *Brachiaria decumbens* pastures defoliated at 3, 4, or 5 leaves per tiller (LT) in two consecutive defoliations (DF).

Variable	DF1			DF2			Main effects					P			
	LT			LT			DF		LT						
	3	4	5	3	4	5	1	2	3	4	5	SEM	LT×DF	DF	LT
Green leaves (% DM)															
IVDDM	71.2 a	66.0 b	63.9 b	62.8	61.7	64.4	67.0 a	63.0 b	67.0	63.8	64.2	1.50	0.006	0.001	0.0800
CP	13.3	13.5	10.1	13.6	10.0	7.7	12.3	10.4	13.5 a	11.7 b	8.9 c	1.51	0.160	0.030	<0.0001
NDF	63.9	66.1	64.4	67.2	66.8	65.4	64.8 b	66.5 a	65.6	66.4	64.9	0.74	0.040	0.003	0.1900
ADF	22.2	25.5	25.2	24.1	27.4	28.2	24.3 b	26.6 a	23.2 b	26.5 a	26.7 a	0.95	0.550	0.002	0.0002
Green stems (% DM)															
IVDDM	62.4	56.3	48.3	60.3	44.4	45.6	55.6 a	50.1 b	61.4 a	50.4 b	46.9 b	4.81	0.22	0.05	0.0004
CP	8.6	6.4	5.4	7.4	5.3	3.5	6.8	5.4	8.0 a	5.8 ab	4.5 b	1.73	0.91	0.13	0.0100
NDF	68.9	74.1	74.1	73.3	74.7	75.0	72.4	74.3	71.1 b	74.4 a	74.6 a	2.26	0.33	0.11	0.0200
ADF	35.3	37.7	39.6	37.5	40.7	40.6	37.6	39.6	36.4 b	39.2 a	40.1 a	3.04	0.85	0.19	0.0020
Dead leaves (% DM)															
CP	10.8	8.4	9.1	9.1	8.3	6.15	9.4	7.8	10.0	8.3	7.6	1.49	0.32	0.06	0.10
NDF	60.9	65.5	67.3	64.4	67.4	68.8	64.5	66.9	62.6 b	66.5 ab	68.0 a	2.77	0.81	0.12	0.02
ADF	33.3	33.2	36.5	35.3	34.5	35.6	34.3	35.1	34.3	33.8	36.1	2.92	0.68	0.59	0.36
Dead stems (% DM)															
CP	5.2	5.4	4.4	4.8	3.3	2.89	5.0 a	3.7 b	5.0 a	4.4 ab	3.7 b	0.88	0.31	0.01	0.03
NDF	75.0	76.2	75.9	76.1	75.6	76.4	75.7	76.0	75.6	75.9	76.1	1.18	0.48	0.58	0.84
ADF	37.3	38.4	38.6	38.9	35.7	37.6	38.1	37.4	38.1	37.1	38.1	1.72	0.17	0.44	0.73

Different letters in the same row indicate statistical differences
DM – Dry Matter (Composition is expressed as a percentage of the dry matter)

CONCLUSIONS

In pastures of *B. decumbens* at the Araucano piedmont, senescence of leaves does not start uniformly in all tillers. However, most of the tillers (65%) present senescent or dead leaves when reaching the sixth leaf, and their nutritional quality decreases. Green stems represent a large portion (43.9%) of the pasture and losses its nutritional quality faster than leaves. Defoliating *B. decumbens* with fewer leaves per tiller, with stems of better quality, improves pasture DM digestibility. However, defoliating the pasture with fewer leaves per tiller (3 leaves) reduces nutrient yield. Therefore, the evidence from this study suggests that *B. decumbens* pastures should be harvested at 4 leaves per tiller.

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Short article

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Behavior of three lettuce cultivars in a hydroponic system

Comportamiento de tres cultivares de lechuga en un sistema hidropónico

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ABSTRACT

The cultivation of lettuce in a hydroponic system is an alternative to optimize the production. Lettuce is an important food, being one of the most consumed vegetables in Brazil. The objective of this study was to evaluate the agronomic behavior of commercial cultivars of lettuce under a single nutritive solution in a hydroponic system. The experiment was carried out under greenhouse conditions. The experimental design was completely randomized, with five replicates. The treatments were composed of commercial lettuce cultivars (Americana Great Lakes, Rafaela-Americana, and Simpson Black Seed). At the time of harvest, the following parameters were evaluated: number of leaves, length of leaves and roots, fresh mass of shoot and root, and stem diameter. The collected data were submitted to an ANOVA. The cv. Rafaela-Americana presented higher leaf length. The evaluated cultivars did not present significant differences in the number of leaves, aerial green mass, green root mass, root length, and stem diameter. The cultivars showed similar variations in the number of leaves, aerial green mass, green root mass, root length, and stem diameter. The cultivar Rafaela-Americana presented a superior performance in the development of leaf length regarding the other cultivars.

Keywords:
Cultivars
Lactuca sativa L.
Nutrient solution
Protected cultivation

RESUMEN

El cultivo de lechuga en un sistema hidropónico es una alternativa para optimizar su producción. La lechuga es un alimento importante, ya que es una de las verduras más consumidas en Brasil. El objetivo de este estudio fue evaluar el comportamiento agronómico de cultivares comerciales de lechuga bajo una única solución nutritiva en un sistema hidropónico. El experimento se realizó en condiciones de invernadero. El diseño experimental fue completamente al azar con cinco repeticiones. Los tratamientos estaban compuestos por cultivares comerciales de lechuga (Americana Great Lakes, Rafaela-Americana y Simpson Black Seed). Al momento de la cosecha se evaluaron los siguientes parámetros: número de hojas, longitud de hojas y raíces, masa fresca de brotes y raíces, y diámetro del tallo. Los datos recopilados fueron sometidos a una ANAVA. El cv. Rafaela-Americana presentó mayor longitud de hoja. Los cultivares evaluados no presentaron diferencias significativas en el número de hojas, masa verde aérea, masa de raíz verde, longitud de raíz y diámetro del tallo. Los cultivares mostraron variaciones similares en el número de hojas, masa verde aérea, masa de raíz verde, longitud de raíz y diámetro del tallo. El cultivar Rafaela-Americana presentó un desempeño superior en el desarrollo de la longitud de la hoja con respecto a los otros cultivares.

Palabras clave:
Cultivares
Lactuca sativa L.
Solución nutritiva
Cultivos protegidos

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Brazil is considered one of the world's largest producers and consumers of vegetables (Santi *et al*., 2010; Pantoja Neto *et al*., 2016; França *et al*., 2017). Its consumption has increased, not only for the healthier and natural food trending, but mainly by the population increase. The consumption market of vegetables has become much more demanding, requiring quality, and supply throughout the year; therefore, production systems have become increasingly specialized (Favarato *et al*., 2017).

In this context, it is necessary to seek better cultivation techniques that overcome this limitation and accomplish with the demand for food. It is important to study cultivation techniques to favor high productivity, coupled with the quality of production and a guaranteed supply of food (Santos *et al*., 2011; Moreira *et al*., 2014). One of the techniques that have aroused a growing interest worldwide is hydroponics. This technique consists in the use of the nutrient solution, in the presence or absence of natural or artificial substrates, providing mineral elements essential to the development of the plant, being applied mainly in vegetable protected crops (Delaide *et al.* 2016; Pantoja Neto *et al*., 2016). It meets the current requirements of production, and in Brazil, its use has expanded, mainly, in the cultivation of lettuce (*Lactuca sativa* L.) because this system presents advantages regarding soil cultivations.

Lettuce (*Lactuca sativa*) is a vegetable belonging to the Asteraceae family, which originated in Europe-Mediterranean. It has high socio-economic importance, contributing to the generation of jobs, as well as food and human health, being a source of many vitamins and minerals and dietary fibers (Ohse *et al*., 2001; Silva *et al*., 2017) and one of the vegetables most commercialized in Brazil (Queiroz *et al*., 2017).

Lettuce stands out in the hydroponic crops of the national scenario, being responsible for up to 80% of this type of production (Alves *et al*., 2011). Hydroponic lettuce cultivation presents advantages for the environment, for the product and the consumer, with the generation of quality products, fast cycle, higher production, besides providing less water and agricultural inputs, among others (Paulus *et al*., 2012). Besides, in protected cultivation, hydroponics is a well-adopted system for lettuce production because it is an intensified production system due to the short cycle crop (Santos, 2017).

For the success of hydroponic cultivation, a fundamental aspect is the choice of the nutrient solution, which must be formulated according to the nutritional need of the plant, containing adequate proportions of the essential nutrients for its growth (Schmidt *et al*., 2001). However, in order to be successful in this system, one of the factors to be considered is the choice of the appropriate cultivar, where the resulting productive potential depends on genetic interaction and the environment. In order to obtain better yields, cultivars adapted to the temperature and photoperiod conditions of the production region must be selected, thus achieving better yields (Brzezinski *et al*., 2017). Thus, the present work had as objective to analyze the production of lettuce under a single nutrient solution in a hydroponics system.

MATERIALS AND METHODS

The experiment was carried out at the Universidade Estadual de Goias (State University of Goiás), in the municipality of Santa Helena de Goiás (17°49'33.9" S, 50°36'224.7" W, average elevation of 570 m). According to the classification of Köppen and Geiger (1928), the climate of the region is type Aw (tropical), with a rainy season in summer and dry season in winter, with an annual average rainfall of 1,539 mm, average air temperature of 24.3 °C. The experimental design was randomized in blocks, and the effects between a single nutrient solution and the cultivars (Americana Great Lakes, Rafaela-Americana, Simpson Black Seed) were studied in five replications.

The experiment was conducted in an arbor, semi-detached, 12 m long, 8 m wide, and 4 m right foot and black shading cover (30% interception of light). The seedlings were produced in expanded polyethylene trays with cells of dimensions 2 cm×2 cm×2 cm with a commercial PlantMax® substrate. Seven days after germination, the seedlings roots were washed, removing any excess substrate from the roots and transplants into the hydroponic system. Soon after the transplant, the treatments were started the same day.

The nutrient solution in all treatments was prepared with the same amount of fertilizers, following the recommendation

of Furlani *et al.* (2009) which contained 750 g of Hydro Special Nitrate Hydro, 500 g of Potassium Nitrate, 150 g of monoammonium phosphate (MAP), 400 g Magnesium Sulphate, 0.15 g of Copper Sulphate, 0.5 g of zinc sulfate, 1.5 g of Manganese Sulphate, 1.5 g of boric acid, 0.15 g of sodium molybdate ($Na_2MoO_{4.2}H_2O$), 30 g of Tenso-Fe® (FeEDDHMA-6% Fe) for every 1000 L of water. The pH of the solution was maintained between 5.5 and 6.5.

The system used was NFT (laminar flow of nutrients), in which the nutrient solution was distributed in the cultivation channels, at a flow rate of 3 L min^{-1} (3W power), with a drive frequency programmed to drive the motor pump for 15 and 30 min disconnected in the daytime period (6 h – 19 h) and for 15 min on, at each interval of 2 h at night (19 h - 6 h).

The nutrient solution was conducted by pumping thorough a PVC tubing (commercial diameter 40 mm) from the reservoir to the top of the bed by a programmed digital timer, returning to the reservoir by gravity. The water used to prepare the nutrient solution was drinking water. For storage of the nutrient solution in the amount of 5 L, transparent plastic tanks with a capacity of 5.5 L and coated with aluminum foil were used to minimize the incidence of solar radiation in the reservoirs.

The nutrient solution was used according to commercial recommendations. According to the recommendations of the surface cultivation, the nutritional solutions were monitored to a pH between 5.5 and 6.5, using the digital pH meter, being the plants with greater availability of nutrients (Carmello and Rossi, 1997; Kopp *et al.*, 2000). ECs were also monitored by a portable conductivity meter, usually to guarantee the results of each treatment. The reading of the electrical conductivity and pH was performed daily to keep them in the electrical conductivity ranges of 1.60 dS m^{-1} and pH between 5.5 and 6.5. The working range of pH and electrical conductivity was determined according to the methodology described by Moraes *et al.* (2018), being the best ranges for pH and electrical conductivity for hydroponics lettuce production. The spacing was 0.25 m between lines and 0.25 m between plants. The plants were harvested when they reached their adult size, and the three central plants of each profile were harvested.

Forty days after planting, weights, and measurements of the following parameters were performed:

- Number of leaves: total leaves were counted in each plant.
- Leaf length (LL) and root length (RL): measurements using a ruler.
- Fresh mass of aerial part (FMA) and root (FMR): separated the aerial part of the root of each plant with a cross-section in the stem and weighed on a precision scale.
- Stem diameter (SD): A precision digital caliper measuring the basal part of the plant was used.

The data were submitted to ANOVA, using the F test, and the Tukey test was performed. The results were considered significant when $P<0.05$ and expressed as mean and standard deviation. All statistical analyzes were performed using the Sirvar software (Ferreira, 2019).

RESULTS AND DISCUSSION

There was a significant difference only for the leaf length variable among the three cultivars. The other variables did not present a significant difference (Table 1).

Table 1. *P*-values for the Analysis of Variance (ANOVA) for the leaf number (LN), fresh mass of aerial part (FMA) and fresh mass of roots (FMR), average leaf length (ALL), root length (RL), and stem diameter (SD), obtained from three lettuce cultivars, evaluated in a hydroponic system in Santa Helena de Goiás, GO.

FV	DG[1]	LN	FMA	FMR	ALL	RL	SD
Cultivars	2	0.9563	0.0690	0.1781	0.0001*	0.3929	0.3766
Blocks	4	0.9069	0.4762	0.2523	0.1538	0.3169	0.3838
Error	8						
CV (%)		13.14	27.45	32.37	5.08	13.52	15.74

*Statistically significant by the F test at the 5% probability level.
[1] Degrees of Freedom

According to this study, no significant differences were observed for most of the parameters evaluated, from the aerial part (leaves and stem) and root (root), although only three cultivars were submitted to the hydroponic production system. Andrade *et al.* (2010), when evaluating ten different lettuce cultivars in the hydroponic production system, identified that some do not present differences in fresh mass weight, and for leaves formed four groups, stem three groups and root only two groups. The results obtained by Andrade *et al.* (2010) differ from this research, but also considers that a much higher number of cultivars were evaluated.

Magalhães *et al.* (2010) found significant differences in fresh shoot weight in the seven lettuce cultivars submitted to different concentrations of nutrient solution in a hydroponic system, highlighting that there were only two groups for this variable, differing from the results obtained in this research. They also highlight the same authors as for the Number of leaves of the cultivars. Vitória Verdinha, Regina 579, and Babá of Summer did not differ from each other but were superior to the others evaluated. These results show that the differences in performance for a number of leaves may not be influenced by the nutrient solution in a hydroponic system, depending on the characteristics of each cultivar.

Gualberto *et al.* (2018) observed significant differences in parameters, number of leaves per plant, stem length, fresh mass, and dry mass between the cultivars Crocantejullie and TPC submitted to different cultivation systems. These results differ from this research considering that no differences were identified between the cultivars Americana Great Lakes, Rafaela-Americana, Simpson Black Seed, so it can be affirmed that cultivars can respond differently to cultivation condition.

The Americana Great Lakes and Simpson Black Seed cultivars had better leaf length averages in relation to the cv. Rafaela-Americana (Table 2). There was a significant difference between the varieties for the average length of leaves of plants on the lettuce cultivars so that the cultivars had different behavior (Table 2), only for cv. Rafaela-Americana, there was a superior effect in relation to the cultivars Americana Great Lakes and Simpson Black Seed in this parameter. The length of leaves is one of the most important characteristics since it is the main one observed by the consumers of fresh vegetables.

In leafy vegetables such as lettuce, the number of leaves is an important characteristic for commercialization since most consumers acquire lettuce per unit and not by weight, taking into account the appearance, volume, and the number of leaves per head (Diamante *et al.*, 2013). The average number of leaves of the three cultivars analyzed was very close, and there was no significant difference (Table 2). These results were lower for the cultivar Simpson Black Seed (curly) when compared to the data obtained by Horino *et al.* (1993), which found an average of 27 leaves per plant considering the cultivars Regina 579, Luisa, Vit. Verdinha, Forest, Summer Nanny, Manoa, Saia Véia.

Table 2. Mean leaf number (LN), fresh mass of aerial part (FMA) and root (FMR), average leaf length (ALL), root length (RL), Stem diameter (SD) obtained from three cultivars of lettuce, evaluated in a hydroponic system in Santa Helena de Goiás, Brasil.

Cultivars	LN	FMA(g)	FMR(g)	ALL(cm)*	RL(cm)	SD(mm)
Americana Great Lake	9.40	33.52	6.34	13.42 b	21.60	7.96
Rafaela-Americana	9.20	37.88	7.08	16.88 a	24.44	8.22
Simpson Black Seed	9.20	23.18	4.60	12.98 b	23.38	7.12
Standard deviation	1.22	8.65	1.94	0.73	3.13	1.22

*Statistically significant variable at 5% according to ANOVA and Tukey test.

The average green air mass of the cv. Rafaela-Americana reached a value of 37.88 g and, close to this value, the Americana Great Lake cv. had an air green mass of 33.52 g. However, the Simpson Black Seed cultivar was not successful, with a green sprout weight of only 23.18 g, but no significant differences were observed (Table 2).

These results were inferior to those obtained by Fernandes (2002) working with lettuce to cultivate summer nanny, Grandes Lagos, and Regina in a conventional hydroponic system, that is, these authors with lettuce cultivars that presented greater weight than the cultivars used in this work.

Da Silva *et al.* (2007) when researching four lettuce cultivars in hydroponic systems, they reported that the cultivar Mônica, from the crespa group, presented higher root development in relation to the cultivar Tainá, from the Americana group while the cultivar Regina, of the smooth group, presented a number of leaves per plant.

The cv. Rafaela-Americana obtained greater development of the root system, consequently obtaining root with higher weights in relation to the cultivars Americana Great Lakes and Simpson Black Seed, and the cv. Americana Great Lakes was superior to the cv. Simpson Black Seed, but there were no significant differences between cultivars (Table 2). The average weight of green roots in the cv. Rafaela-Americana was 7.08 g, while the Simpson Black Simpson had an average of 4.60 g per plant evaluated in the hydroponics system.

There was no significant effect on any of the three cultivars in the root length parameter. The cultivars Rafaela-Americana and Simpson Black Seed had the root length numerically superior to cv. Americana Great Lakes. The average root length of the cultivars Rafaela-Americana and Simpson Black Seed was very close, being 24.44 cm and 23.38 cm of the root, respectively. The cv. Americana Great Lake had a lower mean of 21.60 cm of the root (Table 1 and 2).

The diameter of the stem is an important feature for the fast-food industry, and if the stem is thick, it facilitates the manual removal for later slicing (Blind and Filho 2015). There was no significant difference between the stem diameter variable (Table 1 and 2). It is observed that the highest stem diameter average was in the cultivar Rafaela A, reaching a mean of 8.22 mm, followed by the cultivar Americana Great Leak with an average of 7.96 mm. The cultivar Simpson Black Seed showed a mean stem diameter of only 7.12 mm.

Assessing lettuce production in a hydroponic system in northern Minas Gerais, Aquino *et al.* (2017) worked with four curly cultivars (Verônica, Marisa, Cinderela and Roxane), of the smooth type (Summer Babysitter and Regina) and of the American type (Irene) and concluded that the summer nanny cultivar was the most promising. In our study, the most prominent cultivar was cv. Rafaela-Americana, with greater leaf length.

CONCLUSIONS

The cultivars showed no statistical difference in the number of leaves, aerial green mass, green root mass, root length, and stem diameter. The cultivar Rafaela-Americana presented a superior performance in the development of leaf length regarding the other cultivars.

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The residual effect of metsulfuron on soybean tolerant and non-tolerant to sulfonylureas

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Efecto residual de metsulfurón en soya tolerante y no tolerante a sulfonilureas

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ABSTRACT

Keywords:
Herbicide
Pre-seeding
Safety interval
STS

Metsulfuron is widely used for weed management; however, the residual effect on STS soybean cultivars is unknown. The objective of this work was to evaluate the residual effect of the herbicide metsulfuron on the BMX Garra RR2/STS and M 6410 IPRO (non-STS) soybean cultivars. The herbicide metsulfuron was applied at a rate of 2.4 g a.i. ha^{-1}, in pre-planting of soybean plants. The design was completely randomized in a 2×5 factorial scheme with four replications (first factor: two soybean cultivars - STS and no-STS; second factor: five periods between metsulfuron application and soybean sowing - 0, 15, 30, 45, and 60 days). At 7, 14, 21, and 28 days after sowing (DAS), an emergency evaluation of the soybean seedlings was performed; at 28 DAS, height and dry mass of the shoot were evaluated. The height averages for the STS cultivar were higher than the values of the no-STS cultivar. The average dry mass of soybean plants was higher in the STS cultivar at 0, 15, and 30 days between application and sowing. No differences were observed between the cultivars for the periods of 45 and 60 days, for average dry mass. The BMX soybean cultivar Garra RR2/STS was potentially tolerant for the pre-sowing application of the herbicide metsulfuron. The cultivar of M 6410 IPRO (non-STS) was affected in its initial development by the metsulfuron application during the pre-emergence stage; however, the 60-day metsulfuron application was safer, and therefore, it is recommended to perform the herbicide application in that interval.

RESUMEN

Palabras clave:
Herbicida
Pre-siembra
Tiempo de seguridad
STS

El metsulfurón se usa ampliamente para el manejo de malezas, sin embargo, el efecto residual en los cultivares de soja STS es desconocido. El objetivo de este trabajo fue evaluar el efecto residual del herbicida metsulfurón en los cultivares de soja BMX Garra RR2/STS y M 6410 IPRO (no-STS). El metsulfurón se aplicó a una dosis de 2,4 g i.a. ha^{-1}, en la pre-siembra de soja. El diseño experimental fue completamente aleatorio en un esquema factorial de 2x5 con cuatro repeticiones (primer factor: dos cultivares de soja - STS y no-STS y segundo factor: 5 períodos entre la aplicación y la siembra de soja - 0, 15, 30, 45 y 60 días). A los 7, 14, 21 y 28 días después de la siembra se realizó una evaluación de emergencia de las plántulas de soja, a los 28 días, se evaluó la altura y la masa seca de la parte aérea. Los promedios de altura para el cultivar STS fueron más altos que los valores del cultivar no-STS. La masa seca promedio de las plantas de soja fue mayor en el cultivar STS a los 0, 15 y 30 días entre la aplicación y la siembra. No se observaron diferencias entre los cultivares por los periodos de 45 y 60 días, para el promedio de masa seca. El cultivar de soja BMX Garra RR2/STS fue potencialmente tolerante para la aplicación previa a la siembra del metsulfurón. El cultivar de M 6410 IPRO (no-STS) tuvo su desarrollo inicial afectado por la aplicación de metsulfurón en preemergencia; sin embargo, la aplicación de metsulfurón de 60 días fue más segura y, por lo tanto, se recomienda realizar la aplicación de herbicida en ese intervalo de tiempo.

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Sulfonylurea tolerant soybean (STS®) is not a transgenic crop; it was developed by the technique of seed mutagenesis using the alkylating agent ethyl-methanesulfonate (EMS) (Walter *et al*., 2014). The EMS technique does not cause mutations by insertion into the DNA, but by modifying the already present base by introducing an alkyl radical (Rogozin *et al*., 2001). Mutant seeds from the soybean cultivar 'Williams 82' were selected according to tolerance to herbicide chlorsulfuron of the sulfonylurea group. Thus, the cultivar W20 was developed, which presented a high level of tolerance to some sulfonylureas (Sebastian *et al*., 1989; Walter *et al*., 2014).

Studies have indicated that this characteristic is determined by a semi-dominant allele that has been designated *Als1* and *Als2* (Sebastian *et al*., 1989; Ghio *et al*., 2013; Walter *et al*., 2014; Mantovani *et al*., 2017). The *Als1* allele confers tolerance to chlorimuron, nicosulfuron, rimsulfuron, sulfometuron, thifensulfuron, tribenuron, and flucarbazone while the *Als2* allele confers tolerance to these same herbicides and imazapyr (Walter *et al*., 2014). STS cultivars are highly tolerant to herbicide chlorimuron (Green, 2007; Albrecht *et al*., 2017; Albrecht *et al*., 2018), which can be applied up to four times –the recommended rate for non-STS cultivars (Roso and Vidal, 2011).

Sulfonylureas control mainly dicotyledonous weeds, and some herbicides demonstrate good action against Poaceae and Cyperaceae weeds. Thus, they are widely used to control weeds in soybean, maize, and other crops (Zhou *et al*., 2007).

The herbicide metsulfuron is used in Brazil for crops such as oats, wheat, sugarcane, among other monocotyledons and weed management in winter. The safety interval between application and sowing of soybeans is 60 days (Rodrigues and Almeida, 2018). The application of chlorsulfuron plus metsulfuron, 120 days before sowing (DBS), significantly affected soybean height and yield (non-STS cultivar) (Grey *et al*., 2012). However, there are no reports of the residual effect of metsulfuron on STS cultivars.

It is believed that the safety interval between application and sowing of STS soybeans is smaller than required for non-STS soybeans. The objective of this study was to evaluate the residual effect of herbicide metsulfuron on the BMX Garra RR2/STS and M 6410 IPRO (non-STS) soybean cultivars.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse belonging to the Department of Crop Science of the University of São Paulo, 'Luiz de Queiroz' College of Agriculture, Piracicaba, State of São Paulo (SP), Brazil (22°42'32.0" S, 47°37'43.1" W), from November 2016 to February 2017.

The herbicide metsulfuron (Accurate®, 600 g kg^{-1}, FMC Química do Brasil Ltda., Brazil) was applied at a rate of 2.4 g of active ingredient (a.i.) ha^{-1}, before sowing soybean. The maximum recommended rate for weed control in winter management (off-season) (Rodrigues and Almeida, 2018).

The experiment was conducted in a 2×5 completely randomized factorial design, with four replications. The factors were composed of two soybean cultivars (STS and non-STS) and for five periods between application and sowing of soybean (0, 15, 30, 45, and 60 days). Soybean cultivars BMX Garra RR2/STS and M 6410 IPRO (non-STS) were used. The safety interval between application and sowing of soybeans is 60 days (Rodrigues and Almeida, 2018), which is why the periods chosen were a maximum of 60 days. Therefore, the 60 days are considered the control since it is safe for STS or non-STS soybean.

The experimental units were 5 L pots filled with medium texture soil (Table 1). Before the onset of the experiment, the two cultivars were subjected to a preliminary emergence test, with results higher than 90% at 15 days after sowing (DAS), for both.

At 60, 45, 30, 15, and 0 days, eight pots were applied at a time, four for each cultivar according to the periods between application and soybean sowing. After the application of the last period, the soybean cultivars BMX Garra RR2/STS and M 6410 IPRO (non-STS) were sown with ten seeds per pot, on January 5, 2017.

The applications were performed with a CO_2 pressurized backpack sprayer, bar-equipped with four spray nozzles, at a constant pressure of 200 kPa, a flow rate of 0.65 L min^{-1}, at the height of 50 cm from the target, and a speed of 1 m s^{-1}, with an applied band of 50 cm wide by a nozzle, delivering a spray volume of 200 L ha^{-1}.

Table 1. Result of the chemical and physical analysis of the soil used to fill the experimental units. Piracicaba, SP, Brazil, 2016/17.

pH ($CaCl_2$)	O.M. (g dm^{-3})	Al	H+Al	K	Ca	Mg	SB	CEC	P (resina) (mg dm^{-3})	V (%)
		($mmol_c$ dm^{-3})								
5.3	42	<1.0	25.0	2.6	39.0	16	47.6	66.8	7.0	70
	Clay				Silt				Sand	
	(%)									
	35.0				7.0				58.0	

At 7, 14, 21, and 28 DAS, soybean seedling emergence was evaluated. At 28 DAS, plant height was measured in all the plants in each pot and then averaging the plant height per pot. Also, at 28 DAS, the aerial part of all soybean plants was collected, the plant material was oven-dried at 65 °C for 72 h, and then the dry mass was recorded. The total dry mass per pot and mean dry mass per plant were determined.

Data were tested by analysis of variance and F-test (P<0.05), according to Pimentel-Gomes and Garcia (2002). For the soybean cultivar, the means were compared by Tukey's test (P<0.05) (Tukey, 1949). While for the period, the means were subjected to regression analysis (P<0.05). The Sivar 5.6 program was used for the analysis (Ferreira, 2011).

RESULTS AND DISCUSSION

There is a difference between cultivars in the emergence evaluation at 7 DAS (Figure 1). For the periods 0 and 15 days, between the application and sowing, the cultivar BMX Garra presented superior emergence than the cultivar M 6410 IPRO. For the other periods, no differences were detected between cultivars.

Similarly, differences between cultivars at 0 days were found in the emergence evaluation at 14 DAS (Figure 2). The cultivar STS showed a higher percentage of emergence in this period than the non-STS cultivar; for the other periods, no differences were observed between the cultivars.

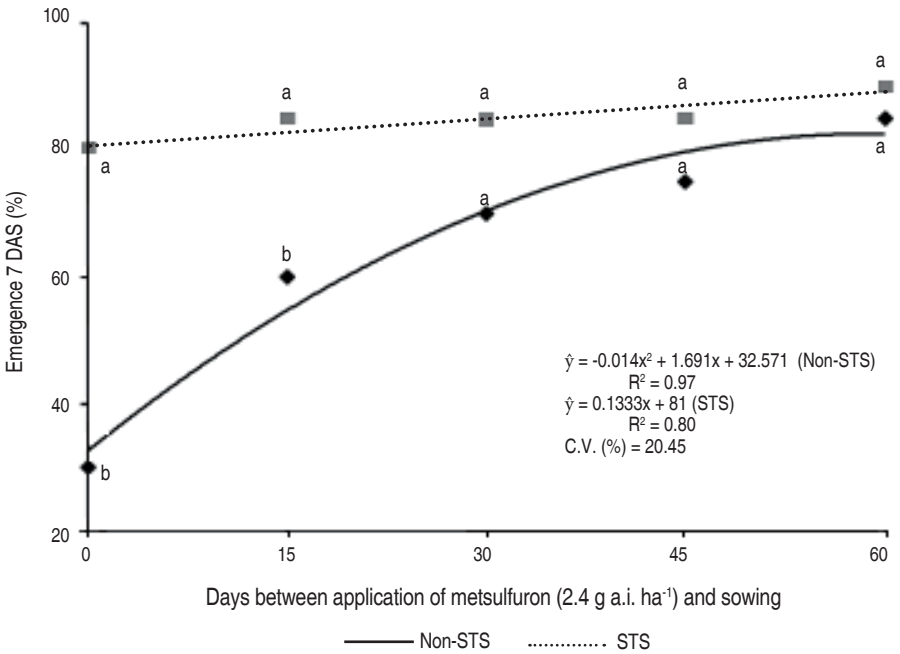

Figure 1. Emergence (%) of soybean plants at 7 DAS, under pre-sowing application of metsulfuron (2.4 g a.i. ha^{-1}). Piracicaba, SP, Brazil, 2016/17. Means with the same letters, comparing cultivars, do not differ by Tukey test (P<0.05).

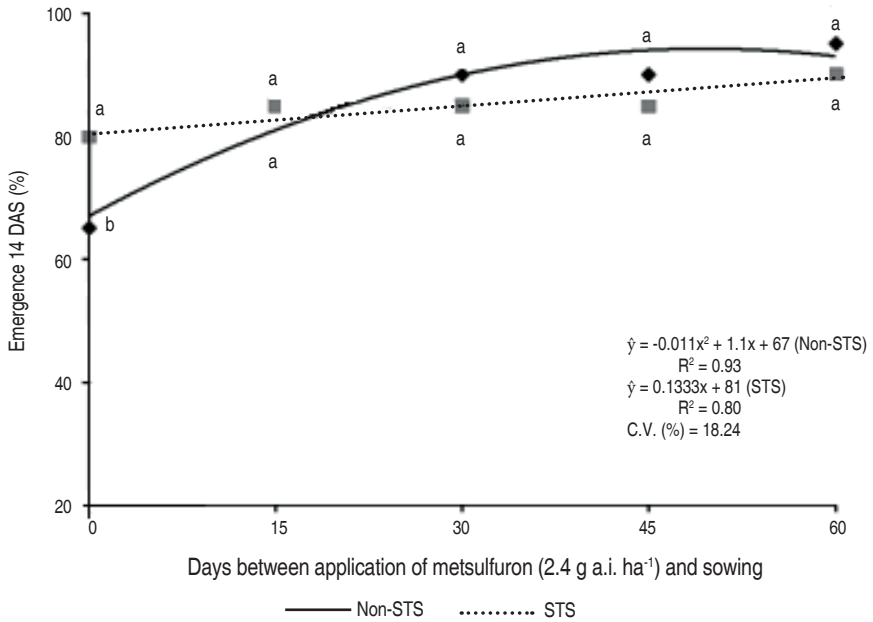

Figure 2. Emergence (%) of soybean plants at 14 DAS, under pre-sowing application of metsulfuron (2.4 g a.i. ha^{-1}). Piracicaba, SP, Brazil, 2016/17. Means with the same letters, comparing cultivars, do not differ by Tukey test (P<0.05).

For the emergence of STS cultivar, at 7 and 14 DAS, it was possible to fit a linear regression; with the decrease of days in the period between application and sowing, the emergence percentage was lower. For the emergence of non-STS cultivar, at 7 and 14 DAS, it was possible to fit a polynomial. Despite the fit, 80.0% emergence occurs at 7 DAS for the STS cultivar (0 d between application and sowing), which is at the recommended minimum limit for seed commercialization (MAPA, 2009). While for the non-STS cultivar, the emergence percentage at 7 DAS was only 30.0% for the same period.

It should be noted that the emergence evaluation was also performed at 21 and 28 DAS (data not shown). However, they were not subjected to statistical analysis since the percentages remained constant from the evaluation at 14 DAS.

For the height of plants (Figure 3), it can be observed that for all periods, values for the STS cultivar were higher than the values of the non-STS cultivar. For the non-STS cultivar, it was possible to fit an upward linear regression, with the increase of days between the application and sowing, the plants' mean height of the increases. While for the STS cultivar, it was not possible to fit a linear regression according to the observed criteria (biological explanation, significant regression, non-significant deviations from regression, coefficient of determination, and residual analysis).

For the variables total dry mass (Figure 4) and mean dry mass (Figure 5), for the STS cultivar, it was not possible to fit a linear regression according to the observed criteria (biological explanation, significant regression, non-significant deviations from regression, coefficient of determination, and residual analysis). It is evidence of the tolerance of the BMX Garra cultivar to the herbicide metsulfuron in pre-sowing, regardless of the period in days, between application and sowing.

However, for the non-STS cultivar, it was possible to fit a linear regression; decreasing the days between application and sowing, the total and mean dry mass of soybean plants also decrease. It should be noted that in the comparison between cultivars, it is not verified difference only for the period of 60 days, for total dry mass. In other periods, the STS cultivar always had the highest total dry mass (Figure 4).

For the mean dry mass of soybean plants, it was observed that, in the comparison between cultivars, the

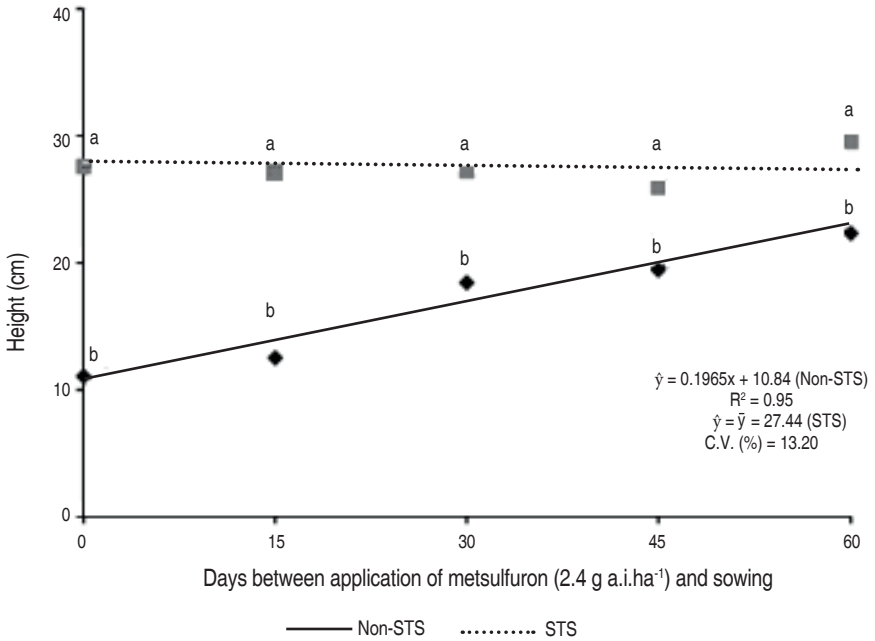

Figure 3. Height (cm) of soybean plants at 28 DAS, under pre-sowing application of metsulfuron (2.4 g a.i. ha^{-1}). Piracicaba, SP, Brazil, 2016/17. Means with the same letters, comparing cultivars, do not differ by Tukey test (*P*<0.05).

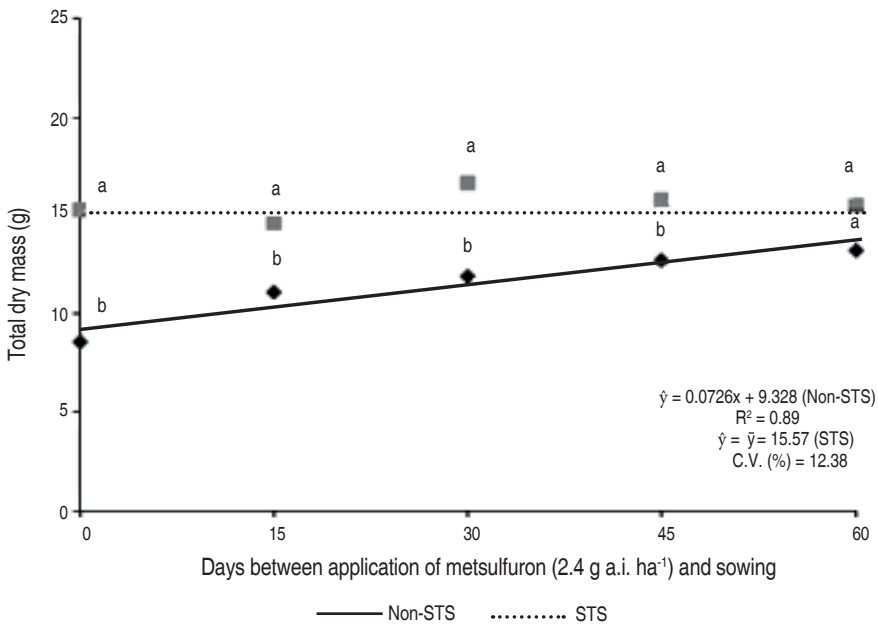

Figure 4. Total dry mass (g) of aerial part soybean plants at 28 DAS, under pre-sowing application of metsulfuron (2.4 g a.i. ha^{-1}). Piracicaba, SP, Brazil, 2016/17. Means with the same letters, comparing cultivars, do not differ by Tukey test (*P*<0.05).

cultivar STS was superior to non-STS for periods of 0, 15, and 30 days between application and sowing. No differences were detected between the cultivars for the periods of 45 and 60 days (Figure 5).

Studies have reported tolerance of STS cultivars for application of the following sulfonylureas: chlorimuron (Poston *et al*., 2008; Silva *et al*., 2016; Albrecht *et al*., 2017; Albrecht *et al*., 2018) and prosulfuron (Anderson

and Simmons, 2004), thifensulfuron (Esbenshade *et al*., 2001), nicosulfuron (Manley *et al*., 2001; Silva *et al*., 2016; Albrecht *et al*., 2017; Silva *et al*., 2018; Silva *et al*., 2019), halosulfuron (Nandula *et al*., 2009), trifloxysulfuron (Porterfield *et al*., 2006), sulfometuron (Piasecki and Rizzardi, 2016) and metsulfuron (Merotto Júnior *et al*., 2000; Albrecht *et al*., 2017). Although there are reports of tolerance to herbicide metsulfuron in some STS cultivars, Silva *et al.* (2016) did not verify tolerance, for post-emergence application, in the cultivar CD 2630 RR/STS.

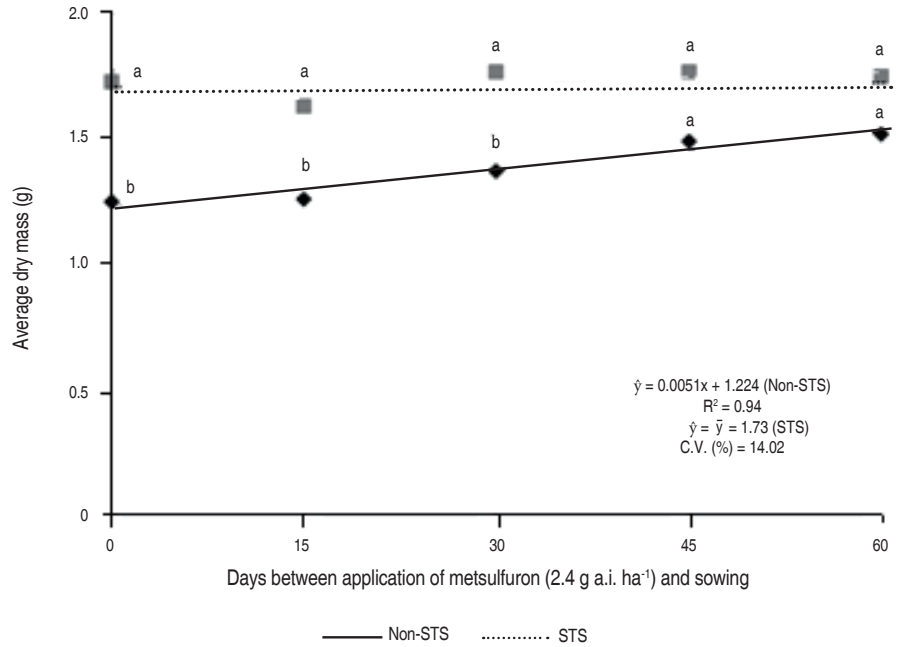

Figure 5. Average dry mass (g) of aerial part soybean plants at 28 DAS, under pre-sowing application of metsulfuron (2.4 g a.i. ha^{-1}). Piracicaba, SP, Brazil, 2016/17. Means with the same letters, comparing cultivars, do not differ by Tukey test (P<0.05).

For non-STS cultivars, the safety interval between application and sowing is 60 days (Rodrigues and Almeida, 2018). In the present study, the cultivar M 6410 IPRO (non-STS) had its initial development impaired by the pre-emergence application of the herbicide metsulfuron. It was possible to fit a regression model for all analyzed variables, reducing the values with a decrease of the days between application and sowing. According to the results, reductions are observed in some STS's variables from 45 days between application and sowing. For the interval of 60 days, in general, no differences are found between the cultivars, also for 60 days, an emergence of 85 and 95% was observed, at 7 and 14 DAS, respectively, for non-STS. Values above of recommended minimum (80%) for seed commercialization (MAPA, 2009).That is, the pre-sowing application of metsulfuron is safe for the non-STS cultivar when the safety interval is respected.

In pre-emergence, there are no reports of selectivity of the herbicide metsulfuron in STS soybean, the results obtained in the present study are of great importance in the positioning of the herbicide metsulfuron in pre-emergence of STS soybean. The results indicate that the cultivar BMX Garra RR2/STS is potentially tolerant of metsulfuron, regardless of the period between application and sowing. Only for the emergence, it was possible to fit a regression; however, even for sowing immediately after application, the emergence percentages were 80.0%.

The herbicide metsulfuron has a broad spectrum of action, controlling mainly eudicotyledons plants and some species of monocotyledons (Rodrigues and Almeida, 2018). The herbicide, alone or in combination, is effective in the control of *Conyza bonariensis* (Vargas *et al*., 2007; Walker *et al*., 2013), *Fimbristylis miliacea*

(Tormena *et al*., 2016), *Hordeum spontaneum* (Izadi-Darbandi and Aliverdi, 2015), *Cirsium arvense* (Zargar *et al*., 2019), among others.

Thus, the herbicide metsulfuron can be used in the management of weeds, especially eudicotyledons plants resistant or naturally tolerant to glyphosate in pre-sowing of STS soybean. In this context, STS cultivars can be of great importance in the management and prevention of the selection of herbicide-resistant biotypes weeds, due to the possibility of using other herbicides (Green, 2007; 2012). As well as the use of the herbicide metsulfuron, in pre-emergence, according to these results. Studies indicate that the use of pre-emergence herbicides, in combination with other management practices, is effective in the control of weeds in soybean crops, as well as in the prevention of the selection of resistant weed biotypes (Neve *et al*., 2018; Rosario-Lebron *et al*., 2019).

CONCLUSIONS

The soybean cultivar BMX Garra RR2/STS, in general, was potentially tolerant to the herbicide metsulfuron, applied at pre-sowing. Therefore, sowing can be done immediately after the application. The cultivar M 6410 IPRO (non-STS) had its initial development impaired by the pre-emergence application of the herbicide metsulfuron. However, when the safety interval recommended (60 days) is respected, the application of the herbicide is safe to the cultivar.

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Physicochemical properties of bean pod (*Phaseolus vulgaris*) flour and its potential as a raw material for the food industry

Propiedades fisicoquímicas de la harina de vaina de frijol (*Phaseolus vulgaris*) y su potencial como materia prima para la industria alimentaria

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ABSTRACT

The sustainable development of agro-industry requires the efficient use of all raw matters, which implies physicochemical and functional studies of by-products for finding their potential usage in industrial processes. The objective of the present study was to assess some physicochemical and functional properties of flour obtained from the bean pod. Two types of drying methods were tested: convection and vacuum. The flours tended to yellow color according to b* parameter values (convection drying: 17.54; vacuum drying: 18.35), with a slight red hue. They also showed high water holding (convection drying: 8.30 g g^{-1}; vacuum drying: 7.56 g g^{-1}) and oil holding capacity (convection drying: 2.53 g g^{-1}; vacuum drying: 2.56 g g^{-1}), polyphenols content (convection drying: 26.62 mg GAE g^{-1} DW; vacuum drying: 3.77 mg GAE g^{-1} DW) and antioxidant capacity (convection drying: 33.42 µmol Trolox g^{-1}; vacuum drying: 5.27 µmol Trolox g^{-1}). The results were similar for the two types of drying methods, except for the antioxidant capacity. Since convection drying is more economical and available than vacuum drying, only the flour obtained by convection method was analyzed on compositional and structural characteristics. This flour showed to be a good source of dietary fiber (66.93%), being the insoluble fiber the predominant fraction (59.97%). Moreover, a high content of ash (6.65%) and low lipid content (0.58%) were also found. The IR spectrum showed signs corroborating the presence of dietary fiber, which also was evidenced morphologically. Overall, the results obtained indicated that the bean pod flour obtained by convection drying has potential use as a raw material for the food industry.

Palabras clave:
Vaina del frijol
Subproductos
Frijol común
Desarrollo
Industria alimentaria
Sostenibilidad

RESUMEN

El desarrollo sostenible de la agroindustria requiere el uso eficiente de las materias primas, lo que implica estudios fisicoquímicos y funcionales de subproductos para encontrarles un uso potencial en procesos industriales. El objetivo del presente estudio fue evaluar algunas de las propiedades fisicoquímicas y funcionales de harina obtenida a partir de la vaina de frijol. Se probaron dos tipos de métodos de secado: convección y al vacío. Las harinas obtenidas exhibieron una tendencia al color amarillo según los valores del parámetro de color b* (secado por convección: 17,54; secado al vacío: 18,35), con un ligero tono rojo. También mostraron alta capacidad de retención de agua (secado por convección: 8,30 g g^{-1}; secado al vacío: 7,56 g g^{-1}) y aceite (secado por convección: 2,53 g g^{-1}; secado al vacío: 2,56 g g^{-1}), contenido de polifenoles (secado por convección: 26,62 mg EAG g^{-1} BS.; secado al vacío: 3,77 mg EAG g^{-1} BS) y capacidad antioxidante (secado por convección: 33,42 µmol Trolox g^{-1}; secado al vacío: 5,27 µmol Trolox g^{-1}). Los resultados fueron similares para los dos tipos de métodos de secado, a excepción de la capacidad antioxidante. Dado que el secado por convección es más económico y disponible que el secado al vacío, solo la harina obtenida por el método de convección fue analizada en sus características de composición y estructurales. Esta harina demostró ser una buena fuente de fibra dietética (66,93%), siendo la fibra insoluble la fracción predominante (59,97%). Además, se encontró un alto contenido de cenizas (6,65%), y un bajo contenido de lípidos (0,58%). El espectro IR mostró signos que corroboraron la presencia de fibra dietética, que también se evidenció morfológicamente. En conjunto, los resultados obtenidos indican que la harina de vaina de frijol obtenida por secado por convección presenta un uso potencial como materia prima para la industria alimentaria.

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Large amounts of waste are generated in the process of food production that can become an environmental and public health problem if they are not adequately treated. Finding new usage for waste is a priority to achieve sustainable development. By-products are those wastes that meet technical specifications and can be used in another production process. By-products of the agri-food industry such as legume pods, fruit husks, and seeds have been considered as potential food by-products because they are a promising source of functional compounds that would satisfy the functional product market and innovate with their presentation (colors and textures) (Estrada-López *et al*., 2018). The transformation of biomass into value-added products is an intensive and continually evolving field of research, that not only affects the food industry but other sectors of the economy (Duque-Acevedo *et al*., 2020).

One of the most important food crops in tropical countries is the common bean (*Phaseolus vulgaris*). It is an essential and economical source of proteins, complex carbohydrates, vitamins, and phenolic compounds (Mojica *et al*., 2015). In 2018, the production of beans in Colombia was 131,716 t while worldwide production was 30.4 million t (FAOSTA, 2018). In the post-harvest management of beans, large amounts of residues are generated. One of them is the pod, which represents an economic and environmental problem due to its high volumes and disposal costs. The pods constitute 39% of the weight of the fruit (Aigbodion, 2019); therefore, the annual production would generate around 84,211 t of the pod in Colombia and 19.4 million t worldwide.

So far, there are no studies in the literature about the properties of the flour from the common bean pod (*Phaseolus vulgaris*). However, some results highlight the importance of the broad bean pod (*Vicia faba* L.) and pea (*Pisum sativum* L.) as sources of fibers (Mateos-Aparicio *et al*., 2010a) and whose flours exhibited functionality in the preparation of baking and confectionery products, like good water holding capacity that positively impacts bread texture (Belghith-Fendri *et al*., 2016a; Martins *et al*., 2017). The preceding reveals the limited exploration of the potential of pulse pods as by-products in the food industry. Other studies have shown that pea pods are used as potential carbon source for bio-butanol production (Nimbalkar *et al*., 2018), and nanofiber of bean pods are proposed as material for automobile applications (Aigbodion, 2019). Pulse pods represent an economical raw material that has been underestimated when used exclusively as fuel or fertilizer (Mateos-Aparicio *et al*., 2010a).

Agro-wastes are materials with high-water content (greater than 80%); therefore, they are perishable. Their utilization depends on the maintenance of their quality as much as possible during storage. One of the ways to meet this need is to carry out a drying treatment to inactivate some enzymes, anti-nutrients and reduce microbiological growth. The drying techniques are also useful to reduce the waste volume to easier lightweight transportation. Some technologies involved are conventional air, microwave, vacuum-freezing, rotary, jet spouted bed, or air jet impingement mechanisms (Yang *et al*., 2020). The convection drying method is the most common and available. By-product as orange peel (Mello *et al*., 2020) and jaboticaba peel (Inada *et al*., 2020) were dried at moderate temperature and showed good properties with this method. Vacuum drying is a technique that exposes the sample to lower temperatures and shorter time, leading to a lesser advance in the detriment of quality properties such as color, aroma, flavor and nutritional value. The vacuum drying was applied to obtain powders from outer cabbage leave (Kuljarachanan *et al*., 2019) and banana peels (Vu *et al*., 2017).

The objective of this research was to obtain common bean pod flour (BPF) using two different drying methods and evaluate its color, water and oil holding capacity, ABTS antioxidant capacity, polyphenol content, and compositional and morphological properties.

MATERIALS AND METHODS

Bean pod flour

Fresh common bean pods (*Phaseolus vulgaris*) were obtained as by-products in a local market in Medellín, Colombia. Samples were selected discarding those with mechanical or physiological damages, then they were washed with deionized water and disinfected with polyhexamethylene biguanide 0.5 mg mL^{-1} (Bigudex) by immersion for 3 min (Mathurin *et al*., 2012).

Two drying methods were applied. One sample was dried by convection method with an air oven (BINDER:

BD 56) at a temperature of 60±2 °C for 8 h; two trays were used with a size of 360×360 mm^2. Alternatively, other samples were vacuum drying in a vacuum drying oven (BINDER: VD 53) at 60±2 °C with a pressure of 5 mmHg for 6 h. Subsequently, the dried samples were ground in an analytical mill (IKA® A1) and sieved in a 60 µm mesh to obtain bean pods flour (BPF). Both BPFs were stored in low-density polyethylene bags for use as needed.

Analysis of color

The color coordinates were measured in a spectrocolorimeter (ColorFlex EZ – HunterLab). Eight grams of sample were weighed in Petri dishes (9 cm in diameter by 1.5 cm in height), and the CIELab coordinates were recorded at room temperature: luminosity (L*), red-green (a*), blue-yellow (b*). Chroma (C*) and tone (*h) parameters were also calculated. The measurements were made in triplicate.

Determination of water holding capacity (WHC) and oil holding capacity (OHC)

WHC was determined by adding 10 mL of deionized water to 1 g of sample; the mixture was dispersed and left to rest for 5 h. Then the dispersions were centrifuged at 2,300 rpm for 30 min. The supernatants were removed, and the centrifuged tube was drained for 30 min to remove the excess of water. The samples were reweighed. WHC was expressed as grams of water held per gram of sample on a dry basis. The OHC was performed using the same steps mentioned above but using canola oil (0.91 g m^{-1} density) instead of water. OHC was expressed as grams of oil held per gram of sample on a dry basis (Chau *et al.*, 1997).

Water activity (A_w) was evaluated using a water activity meter (Pawkit, Decagon Devices, Washington, DC, USA). An average of 2 g of flour was transferred into the sample container to perform the measurements.

Preparation of extract for determination of total polyphenols and antioxidant capacity

The extraction of the antioxidant compounds from the samples was carried out following the method described by Contreras-Calderón *et al.* (2016). 0.5 g of sample was placed in 10 mL capped centrifuge tubes together with 8 mL of a 50/50 v/v methanol-water mixture; subsequently, they were stirred at 280 rpm at room temperature for 1 h. Then, the samples were centrifuged at 5,000 rpm for 10 min, after which the supernatant was filtered and collected in a 25 mL amber volumetric flask. The residue was re-extracted with 8 mL of a 70/30 v/v acetone water mixture, and the centrifugation procedure was repeated. The supernatants were combined in the 25 mL amber volumetric flask. Finally, distilled water was added to make the final volume 25 mL, and they were stored at -18 °C and measured before 24 h.

Analysis of total phenols content (TPC)

The TPC was determined using the Folin-Ciocalteu microscale method described by Rover and Brown (2013). 20 µL of the extract was diluted in 1,580 µL of distilled water and mixed with 100 µL of the Folin-Ciocalteu reagent (Merk) and 300 µL of sodium carbonate solution (Merk). The sample was shaken and kept in the dark at room temperature for 1 h. The absorbance was then measured at 725 nm in the UV-VIS spectrophotometer (Thermo Scientific Evolution 60S). Six aqueous solutions of gallic acid (Sigma-Aldrich Chemical) were prepared at levels concentration ranged from 0 to 500 ppm for the calibration curve. Results were expressed as mg equivalents of gallic acid per g of dried sample (mg of GAE g^{-1} DW).

Analysis of antioxidant capacity (ABTS assay)

The ABTS 2.2'-azinobis (3-ethylbenzthiazoline-6-sulfonic acid) assay was performed following the method proposed by Re *et al.* (1999). Briefly, 100 µL of extract appropriately diluted with water was mixed with 1 mL of $ABTS^{\bullet+}$ radical solution and incubated at 30 °C for 30 min, after which the absorbance was measured at 730 nm (Thermo Scientific Evolution 60S). The calibration curve was constructed with Trolox solutions at concentrations between 0 and 250 µM. The results were expressed as micromoles of Trolox equivalents per g of dried sample (µmol ET g^{-1} DW).

Proximate analysis

The proximate analysis was performed according to the methods of the AOAC (2003): Moisture (method 934.01), protein (method 954.01), fat (method 2.057), ash (method 923.03) and total soluble and insoluble fiber (method 991.42). The carbohydrate content was determined by difference.

Microstructure observation by scanning electron microscopy (SEM)

Analysis of the morphology of the flour obtained was performed by scanning electron microscopy (SEM) using a thermionic microscope in high vacuum drying and secondary electron detector in order to obtain high-resolution images (JEOL JSM 6490 LV). The samples of flours were fixed on a graphite tape and coated with a thin layer of gold (Au) on a Denton Vacuum Desk IV.

Analysis by infrared spectroscopy

In order to molecularly characterize the flour, the FT-IR (Fourier Transform Infrared Spectroscopy) technique was used using an infrared spectrometer (Spectrum One Perkin Elmer Boston USA) at room temperature. KBr pellets were prepared by mixing 1 mg of the sample with 100 mg KBr applying 5 tf pressure with a hydraulic press. The region used was 400 to 4000 $cm^{-1,}$ and commercial pectin was used for comparison purposes.

Statistical analysis

All measurements were performed three times, and the results were expressed as means±standard deviations and reported on a dry matter basis. Analysis of variance (ANOVA) was performed to assess for any significant differences between the means with a level 95% confidence. The Statgraphics Centurion XVII software was used.

RESULTS AND DISCUSSION

The effect of drying method on some functional properties was assessed. Coordinates of CIELab space chroma (C*) and hue angle (h*) values were determined because the color is an important quality characteristic for many products derived from flours such as bread and pasta. The color parameters, shown in Table 1, indicated that the flours tended to yellow color with a slight red hue since both have higher positive b* values and hight h* values. This resulted suggested the presence of yellow pigments. Significant differences (P<0.05) were found in the parameters L*, a* and h* for the flours obtained by the two drying methods. The vacuum sample exhibited the highest luminosity, which was expected since the water evaporates at a lower temperature in less time, causing less browning (Gómez-Narváez *et al.*, 2017). However, the color differences are small. It could be said that the convective drying does not lead to an undesirable color formation in the BPF.

Table 1. BPF properties by convective and vacuum drying.

Parameters	Convection drying	Vacuum drying
L*	36.53±1.31 a	46.96±1.56 b
a*	1.83±0.03 a	2.43±0.16 b
b*	17.54±1.04 a	18.35±0.39 a
C*	17.64±1.04 a	18.51±0.39 a
h*	84.02±0.24 a	82.45±0.52 b
WHC (g g^{-1})	8.30±0.45 a	7.56±0.05 a
OHC (g g^{-1})	2.53±0.15 a	2.56±0.06 a
A_w	0.17±0.40 a	0.15±0.30 a
Antioxidant Activity (µmol ET g^{-1} DW)	33.42±2.07 a	26.62±1.72 b
TPC (mg GAE g^{-1} DW)	5.27±0.42 a	3.77±0.30 b

The values are expressed as the mean±standard deviation (n=3). Different letters among the columns mean statistical differences (P<0.05).

There have not been previous studies about color parameters for bean pod flour or powder. The results obtained are comparable to those found in wholemeal flours (Hidalgo *et al.*, 2017) and unconventional flours such as dietary fiber powder obtained from the hull of soybean that presents values of L*=58.55, a*=-2, and b*=19.6 (Yang *et al.*, 2014).

The determination of functional properties such as WHC and OHC are important to define the functional profile of the flour and its possible application. Table 1 shows the values of these two functional properties for BPF. The results did not vary significantly between the drying methods.

The value of WHC allows determining the degree of interaction with water. The flours obtained by the two drying processes showed high values of WHC concerning those found in both pea flour (3.69 g g^{-1}) and broad bean (4.46 g g^{-1}) (Belghith-Fendri *et al.*, 2016a); as in the fiber extracted from these two legumes of 4.6 g g^{-1} and 6.98 g g^{-1}, respectively (Belghith-Fendri *et al.*, 2016b). These results also approximate the values reported by Mateos-Aparicio *et al.* (2010a), who evaluated the WHC of polysaccharides isolated from the pea pod and broad bean, finding values of 8.96 g $g^{-1}$and 9.96 g g^{-1}, respectively. Besides, the WHC values obtained for BPF were high in comparison to those reported for grapefruit, lemon, orange, and apple by-products (1.6-2.3 g g^{-1}) (Figuerola *et al.*, 2005); also for those recently reported by Masli *et al.* (2018) for by-products of diverse botanical origin whose ranges of variation were from 1.3 to 3.9 g g^{-1}, and they are even higher concerning wheat bran (2.7-3.6 g g^{-1}) (Caprez *et al.*, 1986).

The high value of WHC suggests that BPF could be used in products that require hydration processes viscosity or texture development (López-Vargas *et al.*, 2013) in physical stress production processes such as kneading extrusion and homogenization. It also helps to avoid syneresis phenomena during storage (Masli *et al.*, 2018).

Regarding OHC the values found in the present study are high compared to those reported by Belghith-Fendri *et al.* (2016a) in pea and bean pod flours with values of 1.14 g $g^{-1}$and 1.42 g g^{-1}, respectively; and those found for the isolated polysaccharides of pea (0.28 g g^{-1}) and broad bean (0.13 g g^{-1}) (Mateos-Aparicio *et al.*, 2010a). They are also greater than orange by-products (2.15 g g^{-1}) (Fernández-López *et al.*, 2009), cauliflower fiber (0.5 g g^{-1}) (Femenia *et al.*, 1997) and wheat bran (1.5 g g^{-1}) (He *et al.*, 2018). However, they are smaller compared with fibers from the rice husk (Sangnark *et al.*, 2004), bagasse from native sugarcane (3.26 g g^{-1}), and asparagus (5.28-8.53 g g^{-1}) (Elleuch *et al.*, 2011).

The OHC is related to surface properties, density porosity size, and hydrophobic nature of the fiber particle (López-Vargas *et al.*, 2013). The values found suggest that BPF could be used for the formulation of emulsions and high-fat food products to improve their physicochemical stability as well as to improve the retention of aromas.

Water activity is an important indicator of flours because it affects their shelf-life. It is important to highlight that A_w values of the BPF obtained by both processes indicated are chemically and microbiologically stable.

The results for the antioxidant capacity by ABTS and total phenols content (TPC) are observed in Table 1. Significant differences were found (P<0.05) between the different drying methods, the flour obtained by convective drying presented greater antioxidant capacity and TPC, which was not expected because bioactive compounds such as polyphenols are susceptible to degradation when subjected to thermal treatment (Cárcel *et al.*, 2019). Therefore, it could be possible that the highest antioxidant activity obtained in this flour may be due to compound formed during the Maillard reaction (Contreras-Calderón *et al.*, 2016; Chua *et al.*, 2019), which can be evidenced with the lower luminosity of flour obtained by convection drying (Table 1).

Both flours exhibited a TPC higher than those found in pineapple by-products (3.74 mg GAE g^{-1} DW), banana (3.85 mg GAE g^{-1} DW), papaya (3.15 mg GAE g^{-1} DW), watermelon (1.46 mg GAE g^{-1} DW), melon (1.49 mg GAE g^{-1} DW), and passion fruit 0.86 mg GAE g^{-1} DW). They were lower regarding by-products from the avocado peel (12.52 mg GAE g^{-1} DW) (Morais *et al.*, 2015). Antioxidant capacity and TPC in bean pod have not been reported previously.

The high TPC found in BPF was an expected result since phenolic compounds tend to accumulate in the dermal tissues of plants to fulfill a fundamental role in photoprotection against ultraviolet radiation and the defense against pathogens and predators (Gharbi *et al.*, 2017). On the other hand, foods with antioxidant properties are highly valued due to potential benefits in health. Nowadays, the role of food rich in antioxidants in the preventive effect on degenerative diseases is a field of research that has attracted great attention (Contreras-Calderón *et al.*, 2016). Therefore, BPF has a high potential to be used in the development of functional foods or nutraceutical products.

Altogether these results show that the convective drying method is a good alternative for obtaining BPF, which allows preserving its functional properties such as color, WHC, OHC, and antioxidant. Moreover, the convective drying method is an economical and most available method

than the vacuum drying method. Therefore, in order to gain insight into the compositional, morphological, and structural characteristic of BPF were performed only in the sample obtained by convection drying.

The proximate analysis of the BPF expressed in g per 100 g dry base gave the following results: Humidity (7.77±0.08%), ash (6.65±0.08%), protein (2.42±0.01%), fat (0.58±0.00%), total dietary fiber (66.93±1.01%), insoluble dietary fiber (59.97±0.68%), soluble dietary fiber (6.96±0.58%) and carbohydrates (15.65%).

The moisture content was found below the maximum limit (15.5%) according to the CODEX standard for wheat flour (Codex Alimentarius, 1985), suggesting a good behavior under storage.

The results show that BPF has a higher percentage of fiber compared to the percentages of total, insoluble and soluble fiber reported for the pea pod (58.6%, 54.4% and 4.2%, respectively) and the broad bean pod (40.1%, 30.8%, and 9.3%, respectively) by Mateos-Aparicio *et al.* (2010b). However, the percentages of ash protein and fat were lower than those reported in pea pod (8.07%, 13.37%, and 1.06%, respectively) and bean (9.02%, 13.43%, and 0.24%) by Belghith-Fendri *et al.* (2016a) and also against those found by Mateos-Aparicio *et al.* (2010b) in pea pod (6.6%, 10.8%, and 1.3%) and broad bean (6.3%, 13.6%, and 1.3%).

The results found, highlight the importance of BPF as a source of fiber minerals and low lipid content.

Regarding morphological analysis, the micrographs show fibrillar fragments with irregularly shaped (Figure 1A), which referred to the high fiber content found in BPF. At higher magnification, the presence of microfilaments aligned in parallel along the particles is observed (Figure 1B). A closer approach to the particles shows that they are porous structures (Figure 1C). A similar morphology has been found in the pod of other edible leguminous such as *Parkia speciosa* (Gan and Latiff, 2011) and rice husk (Sánchez-Safont *et al.*, 2018). The porous morphology supports the high values found in WHC and OHC (Table 1).

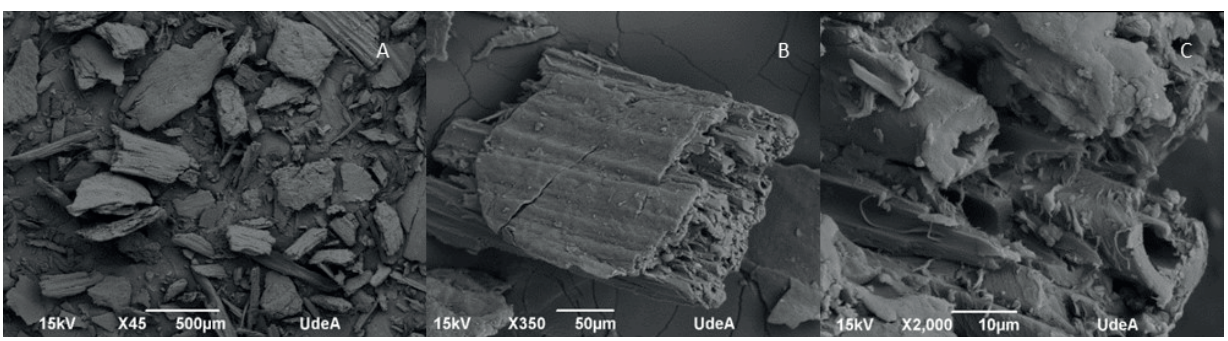

Figure 1. Micrographs of BPF convective drying method in different magnifications A. 45x; B. 350x; C. 2000x.

IR spectroscopy is an excellent tool for the analysis of carbohydrates, and since BPF had a high content of these compounds, its IR spectrum was analyzed. Figure 2 shows the spectrum of BPF compared to the spectrum of commercial citrus pectin. BPF spectrum exhibited strong absorption bands that coincide with characteristic signals of the dietary fibers.

The characteristic signals found were broadband at 3400 cm^{-1} associated with the OH stretch. The 2950 cm^{-1} signal due to the stretching of the methyl group. The lengthening at 1750 and 1650 cm^{-1} indicated the presence of esterified and free carboxylic groups, respectively. The peaks at 1420 -1380 $cm^{-1}$are assigned to C-H deformation vibrations. The signals in the region comprised between 1200-900 cm^{-1} are ascribed to C—O, and C—C stretching vibrations of the polysaccharide molecules. The 600 cm^{-1} signal is associated with C-C-C in-plane bending (Arroyo Salas *et al.*, 2008; Kamarudin *et al.*, 2016). The signals are congruent with cellulose and pectin type fibers, as previously was stated by Stolle-Smits *et al.* (1999).

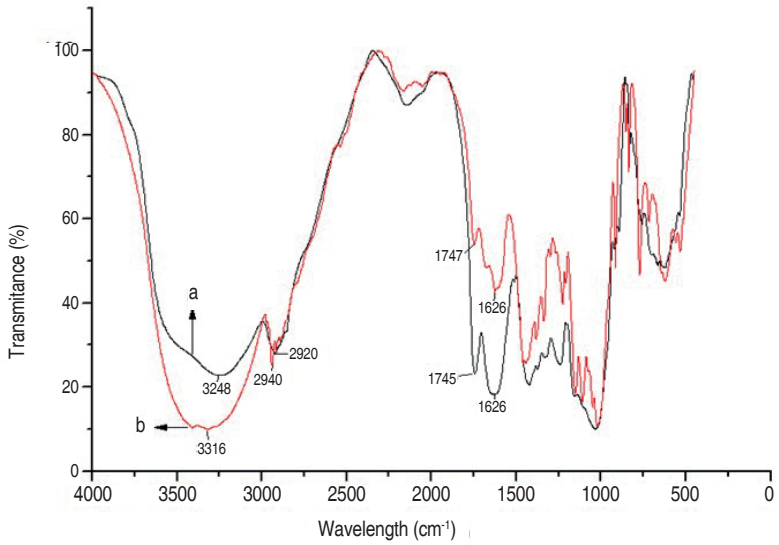

Figure 2. IR spectrum with Fourier transform of A. commercial citrus pectin and B. BPF by convective drying method.

CONCLUSIONS

This study provides practical information for the use of the bean pod as a by-product for obtaining flour. The properties of color, water and oil holding capacities were similar for both types of flours from convection and vacuum drying. ABTS antioxidant capacity and polyphenol content were higher when pods were dried by convection. Additionally, it showed that flour obtained by convective drying has a high content of fiber, minerals, and low lipid content. Overall, the results indicated that BPF has considerable potential to be used in the food industry, being interesting for the development of functional foods and value-added products.

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Physicochemical properties, sensory attributes and consumer preference of soursop leather

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Propiedades fisicoquímicas, atributos sensoriales y preferencias del consumidor de un laminado de guanábana

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ABSTRACT

A soursop leather was prepared, and its physicochemical and sensory properties were assessed. The preparation of the leather was carried out based on an experimental mix design. The combination of soursop pulp (79-100%), sugar (0-20%), and citric acid (0-1%) produced five treatments. The optimum mixture, viz., T_{II} (80:20:0), and the midpoint mixture, viz., T_{V} (89.5:10:0.5), were finally selected, using an acceptability test (taste and color) with an untrained panel. These two treatments were evaluated, recording the variation of total soluble solids (TSS), pH, titratable acidity, ascorbic acid, total polyphenols, and color (chroma, hue angle, browning index and total color difference) every 15 days for a 45-day period. It was determined that it can be prepared from the soursop pulp, a leather with high acceptability (taste; color): T_{IV} (8.68; 7.90) and T_{II} (8.51; 7.72) on a 12-point scale. Significant changes in TSS, pH, titratable acidity, total polyphenols, and color (chroma, browning index, and total color difference) were observed during the 45 days of storage at room temperature. Both ascorbic acid and total polyphenol content make the soursop leather a product with potentially healthy characteristics.

Palabras clave:
Aceptabilidad
Annona muricata L.
Laminados de frutas
Productos frutales
Alimento saludable

RESUMEN

Se preparó un laminado de guanábana para evaluar sus propiedades fisicoquímicas y sensoriales. La elaboración del laminado se realizó partiendo de un diseño experimental de mezcla. La combinación de pulpa de guanábana (79-100%), azúcar (0-20%) y ácido cítrico (0-1%) produjo cinco tratamientos de los que fueron seleccionados dos, mediante una prueba de aceptabilidad (sabor y color) con un panel no entrenado. Se escogieron el T_{II} (80:20:0), que resultó ser la formula óptima, y el T_{V} (89,5:10:0,5), el punto medio. Estos dos tratamientos se evaluaron durante 45 días, registrando cada 15 días la variación de sólidos solubles totales, pH, acidez titulable, ácido ascórbico, polifenoles totales, y color (croma, tono, índice de amarronamiento y diferencia total de color). Se determinó que a partir de la pulpa de guanábana se puede preparar un laminado de elevada aceptabilidad (sabor; color): T_{IV} (8,68; 7,90) y T_{II} (8,51; 7,72) en una escala de 12 puntos. Se observaron cambios significativos en SST, pH, acidez titulable, polifenoles totales y color (croma, índice de pardeamiento y diferencia total de color) durante los 45 días de almacenamiento, a temperatura ambiental. El contenido de ácido ascórbico y de polifenoles totales hacen del laminado de guanábana un producto con características potencialmente saludables.

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One of the aspects that human beings have neglected in recent times is their diet. When opting for easy access versions such as the "fast food" offers, they fail to consume the daily intake of nutrients, thus affecting the proper functioning of their bodies in the mid- and long-terms. The various consequences of that kind of diet are the increasing incidence of chronic diseases such as diabetes, metabolic syndrome, cardiovascular diseases and cancer, or those due to malnutrition such as obesity (Sastre, 2010). A pleasant and accessible way that could contribute to the daily intake of nutrients, while preventing the incidence of diseases, is the consumption of fruits and their products (Martínez-Navarrete *et al*., 2008; Suna *et al*., 2014).

Fruits are either ingested fresh or through versatile preparations, rich in nutrients, minerals, fiber, carbohydrates, water, vitamins, and antioxidants (Barret, 2007; Patel *et al*., 2016). Tropical fruits, in general, have high acceptability due to their exotic aromas and flavors. Among these, the soursop is a fruit rich in minerals (Fernández *et al*., 2007), vitamins (Ojeda *et al*., 2007), and there is evidence that because of its bioactive content, it can be considered a food with potential healthy characteristics (Ramírez and Pacheco, 2011; Ávila de Hernández *et al*., 2012; Vit *et al*., 2014). Unfortunately, it is an underutilized crop with a short postharvest life (Pinto *et al*., 2005). This fruit continues ripening once it is harvested due to its climacteric character, showing pulp softening and fragility in the bark, resulting in difficult handling. The latter exposes it to deterioration by microorganisms, leading to it being easily discarded (Pareek *et al*., 2011). Postharvest losses account for 78.8% concerning the quantity of harvested fruit because of the poor management of pests and diseases, as well as their perishable nature (Arauz and Mora, 1983).

In this regard, the preparation of soursop leathers offers a new option. Fruit leathers are the product of the dehydration of fruit pulp, or a mixture of them, to which preservatives and sweeteners can be added. The result is then molded into a sheet and left to dry until it acquires a textile appearance (Khan A *et al*., 2014). Besides, with the worldwide increase in demand for dehydrated products, coupled with population growth and transport costs, dehydrating fruit pulp generates a stable product with a beneficial quality-volume ratio, long shelf life, low packing cost, and a lower handling weight, while concentrating the original flavor, and maintaining the nutritional quality of many agricultural products (Khan M *et al*., 2014).

Fruit leathers have been prepared from a wide variety of fruits, including guava (Ashaye *et al*., 2005; Khan M *et al*., 2014), mango (Vanegas and Parra, 2012; Hernández-Varela *et al.* 2013), apricot (Suna *et al*., 2014), and papaya (Ashaye *et al*., 2005; Hernández *et al*., 2012; Sujatha and Sayantan, 2014). Mixtures have also been made, namely, apple-quince (Torres *et al*., 2015), kiwi-apple (Diamante and Dong, 2015), mango-papaya (Bhalerao *et al*., 2017), apple-banana (Parimita and Puneet, 2015), among others. Although soursop pulp has been mixed with katuk leaves puree (Utomo *et al*., 2014), there is no evidence of the preparation and valuation of the quality of leather made with this single fruit. Therefore, the purpose of this research is to prepare and evaluate the physicochemical and sensory properties of a soursop leather during 45 days of storage at room temperature.

MATERIALS AND METHODS

The research was conducted in the Laboratory of Horticultural Products, Deanship of Agronomy, Universidad Centroccidental Lisandro Alvarado, Agua Viva-Venezuela.

Selection and preparation of fruit samples

Soursops were purchased, at physiological maturity, from a local supermarket in Barquisimeto, Lara State. They were chosen randomly according to their physical aspect, and the selection parameters were (i) do not present physical damages (bruises, cuts), neither by pests nor pathogens; (ii) preserve the peduncle; (iii) be firm to the touch. Once in the laboratory, whole fruits were first washed with running water at room temperature (28 °C) for removing dust and foreign materials; a second wash was carried out with chlorinated water (5 g per 100 mL) for sanitation. Finally, the soursops were dried with absorbent paper and left, at laboratory environmental conditions, until they achieved the organoleptic maturity to manual peeling, de-seeding, pulping, and freezing (-20 °C).

Preparation of soursop leather

The soursop pulp was left overnight in a refrigerator for defrosting; then, with a laboratory blender (General Electric), the pulp was mixed with a little potable water to improve the mix. Next, this mixture was pasteurized in a Memmert thermal bath (80 °C) and left at room temperature for cooling. The fruit leather was prepared according to the formulas shown in Table 1, spread in Teflon trays with a nonstick surface (38 cm×23 cm×1 cm), and dried in a Memmert stove (60±2 °C) during 6-8 h, until translucent and slightly tacky to the touch, with a final moisture content between 1-7%. The soursop leather formulas were chosen by an experimental design of mixtures (Salamanca Grosso *et al.*, 2015), and the components of the mixtures, ranging from 0 to 100% Wet Base (WB). (min-max). Soursop was 79-100%, sugar was 0-20%, and citric acid was 0-1%. After drying, the trays were left at room temperature to cool down; the fruit leathers were removed from the trays, cut with a kitchen scissor in rectangles (13 cm×20 cm), and packed in cellophane thermo-sealed bags until analyses.

Table 1. Soursop leather formulations.

Treatment	Ingredients content (% WB)		
	Soursop pulp	Sugar*	Citric acid
I	100	0	0
II	80	20	0
III	99	0	1
IV	79	20	1
V	89.5	10	0.5

* Commercial sucrose.
WB: Wet Base

Sensory evaluation

An effective acceptability test was applied to choose the two best treatments. This selection was performed based on taste and color acceptability of the leather, through an untrained panel of 60 potential consumers (57% female) aged from 17 to 55 years. The samples consisted of squares pieces of the product (2×2 cm), which were served in disposable trays, and all leathers were tested at the same time. The sensory ballot had a continuous linear non-structured hedonic scale. The line between *"dislike extremely"* and *"like extremely"* had a total length of 12 cm, with a midpoint to indicate indifference. Also, the ballot included a question related to the intention of purchase the product. The tastings were made from 8:00 to 11:00 am to avoid any physiological state (hunger, fatigue) that could disturb the response of the panelist. The room was a well-lit and ventilated place, without any source of noise or odors that could distract the attention of the judges.

Assessment of physicochemical characteristics

Once selected the two best treatments, the storage of the soursop leather was carried out at room temperature of 23-30 °C (min-max), with a relative humidity of 31-66% (min-max). The evaluation was performed for 45 days, recording the variation of total soluble solids (TSS), pH, titratable acidity, ascorbic acid, total polyphenols, and color (chroma value, hue angle, browning index and total color difference) with time, starting from a zero time established as the initial reference parameter, and every 15 days.

The pH was determined with a Fisher Scientific Accumet Research AR10 potentiometer (COVENIN, 1979). TSS, by refractometry with an Abbe Refractometer Model 1T (ATAGO, Co., LTD), the results were expressed in Brix degrees (COVENIN, 1983). The titratable acidity was determined by potentiometric titration (COVENIN, 1977), and was expressed in function of malic acid concentration (ICONTEC, 2003). Vitamin C, with the dichlorophenol-indophenol method (COVENIN, 1982). The total phenolic content was performed by a Colorimetric Method using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965 cited by Kalt *et al.*, 1999). The total phenolic content was determined by a gallic acid (Scharlau) calibration curve 0-200 mg L^{-1}, at 765 nm

(*y=0.01018x+0.10579; R^2=0.98*), and expressed in mg gallic acid per 100 mg fruit leather (mgGAE 100 mg^{-1}).

The color was quantified by a HunterLab ColorFlex color measurement instrument (HunterLab, Reston, VA). The Chroma value (Equation 1), Hue angle (Equation 2), the total color difference (ΔE) (Equation 3) and browning index (BI) (Equation 4), were calculated from the Hunter L* (whiteness or brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness) -values. The ΔE is a single value which considers the differences between the L*, a*, and b* of the sample and standard (subscript "$_o$" refers to the color reading of soursop leather at zero time) (HunterLab, 2008); and BI, describes the color change during storage or processing (Maskan, 2001).

$$Chroma=(a^2+b^2)^{0.5} \quad (1)$$

$$Hue\ angle=tan^{-1}\left(\frac{b}{a}\right) \quad (2)$$

$$\Delta E=\sqrt{(L_0-L)^2+(a_0-a)^2+(b_0-b)^2} \quad (3)$$

$$BI=\frac{100(x-0.31)}{0.17},\ x\frac{(a+1.75L)}{(5.645L+a-3.012b)} \quad (4)$$

Statistical analysis

The evaluation of the acceptability of the treatments, and the effect of each component in the fruit leather formula, was assessed on the final properties (taste, color) and intention of purchase of the product; then, this was considered on the experimental design of mixture with the software JMP® 9.0 (SAS® Institute Inc., USA). Mean values, standard deviation, and significance test of all the parameters were computed using Origin Pro 8.0 (OriginLab Corp., USA).

RESULTS AND DISCUSSION

Sensory evaluation

Table 2 shows the average response values of the experimental design of mixtures, used to determine the effect of the concentration of soursop pulp, sugar, and citric acid on the acceptability (taste, color), and intention of purchase of the soursop leathers. The acceptability of the evaluated attributes, taste and color, were found in a range from 3.753 to 8.709 and 5.815 to 8.066, respectively. These values are higher than those reported for apple:banana pulp fruit bar supplemented with omega-3 fatty acid (Parimita and Puneet, 2015), peach pulp:soybean slurry leathers (Anju *et al.*, 2014) or soursop pulp:katuk leaves puree leathers covered with chocolate (Utomo *et al.*, 2014). It could be considered that preparing a leather with fewer ingredients, promotes the acceptability of the product because it has a more fruity taste. The intention of purchase was between 16.67 and 78.33%.

Table 2. Average response values of the experimental design.

Treatment	Ingredient content (%WB)			Acceptability		Purchase intention (%)
	Soursop pulp	Sugar	Citric acid	Taste	Color	
I	100.0	0	0	4.518	6.429	25.00
I	100.0	0	0	4.979	6.218	23.33
II	80.0	20	0	8.417	7.368	76.67
II	80.0	20	0	8.613	8.066	76.67
III	99.0	0	1	3.816	5.888	16.67
III	99.0	0	1	3.753	5.815	21.67
IV	79.0	20	1	8.653	7.928	76.67
IV	79.0	20	1	8.709	7.867	78.33
V	89.5	10	0.5	6.411	6.804	48.33
V	89.5	10	0.5	6.903	7.369	60.00
V	89.5	10	0.5	7.338	6.872	51.67

WB: Wet Base

The sugar and soursop pulp contents had a significant effect (P<0.05) on the taste, color acceptability, and the intention of purchase of the soursop leathers (Table 3). A higher percentage of sugar led to higher acceptability of taste, color, and intention of purchase. Whereas the pulp content had an opposite effect, a higher pulp content, resulted in lower acceptability of taste, color, and intention of purchase. Similar results were presented by Utomo *et al.* (2014), who prepared a fruit leather to evaluate the acceptability of different soursop pulp:katuk leaves puree, covered by chocolate. They found that the acidity of the leather was proportional to the proportion of soursop pulp in it, resulting in a decrease of the acceptability by the panelists. This rejection is due to a bitter taste in the soursop, caused by its organic acids (malic acid, citric acid, and isocitric acid) present in this fruit (Ashari 2006 cited by Utomo *et al.*, 2014). Citric acid, on the other hand, had no significant effect on the response variables studied (P>0.05), as observed in the prediction profile graph (Figure 1).

Table 3. Test of the effects of the variables soursop pulp, sugar, and citric acid.

Source	N	DF	Taste			Color			Purchase intention		
			Sum of squares	F-value	Prob>F	Sum of squares	F-value	Prob>F	Sum of squares	F-value	Prob>F
(Pulp-0.79)/0.21	1	1	61.7783	300.8188	<.0001*	111.9882	1310.705	<.0001*	1659.876	95.776	<.0001*
Sugar/0.21	1	1	227.3943	1107.2570	<.0001*	173.6389	2032.264	<.0001*	18303.016	1056.100	<.0001*
Citric acid/0.21	1	1	0.0683	0.3325	0.580	0.0462	0.541	0.4830	1.891	0.109	0.749

Note. N: number of parameters; DF: degrees of freedom

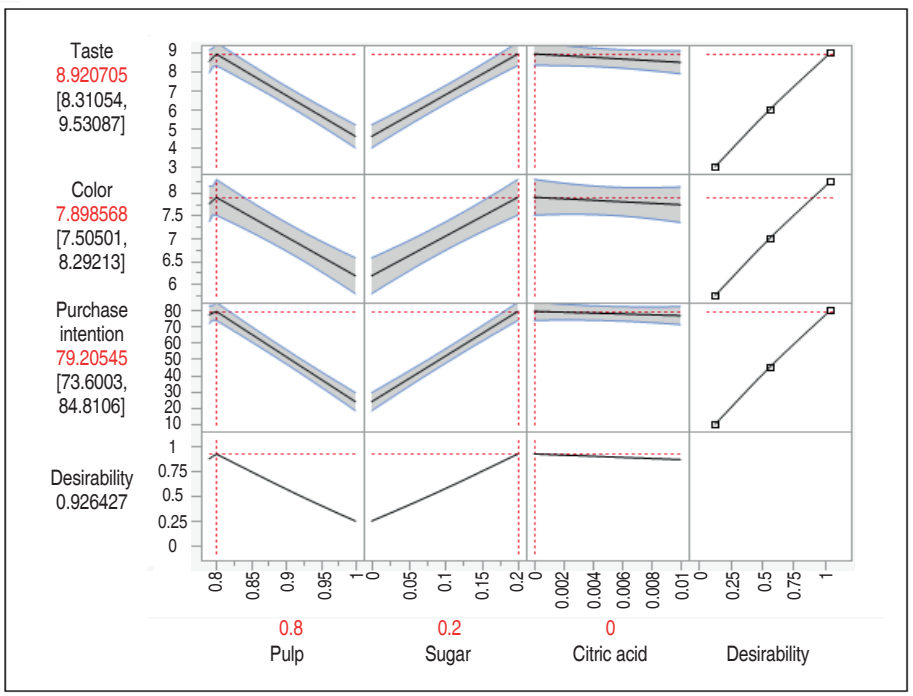

Figure 1. Prediction profile according to the soursop leather ingredients.

The balance between sweetness and tartness in fruit products is important to promote a pleasant taste, and it has a direct impact on acceptability. In this sense, as the soursop pulp has high acidity, in relation to other fruit pulps, it requires more sugar to balance the taste. Hence, the sugar content has significantly increased the acceptability of the taste of the soursop leathers.

However, if it is sought to increase the acceptability of the product, adding more sugar to the formula would undermine the healthy nature of fruit leathers. Otherwise, it is possible that the concentration of citric acid did not significantly affect the response variables because of the levels tested in this study. The optimal proportions of the ingredients that simultaneously maximize the acceptability of taste, color, and intention of purchase, were obtained by maximizing the desirability function (Figure 1). A maximum value of 0.926 was found for this function when the soursop leather is prepared with 80% pulp and 20% sugar, resulting in the optimum mixture.

All the linear models for each response variable studied (Figure 2), were significant ($P \leq 0.001$), with coefficients of determination (R^2) greater than 90%, showing a good fit. On the other hand, the estimated coefficients for these models were also significant ($P \leq 0.001$), except those associated with the concentration of citric acid, which was expected because this factor was not significant for acceptability (taste, color), or intention of purchase. Consequently, the terms associated with it were excluded from the adjusted models (Table 4).

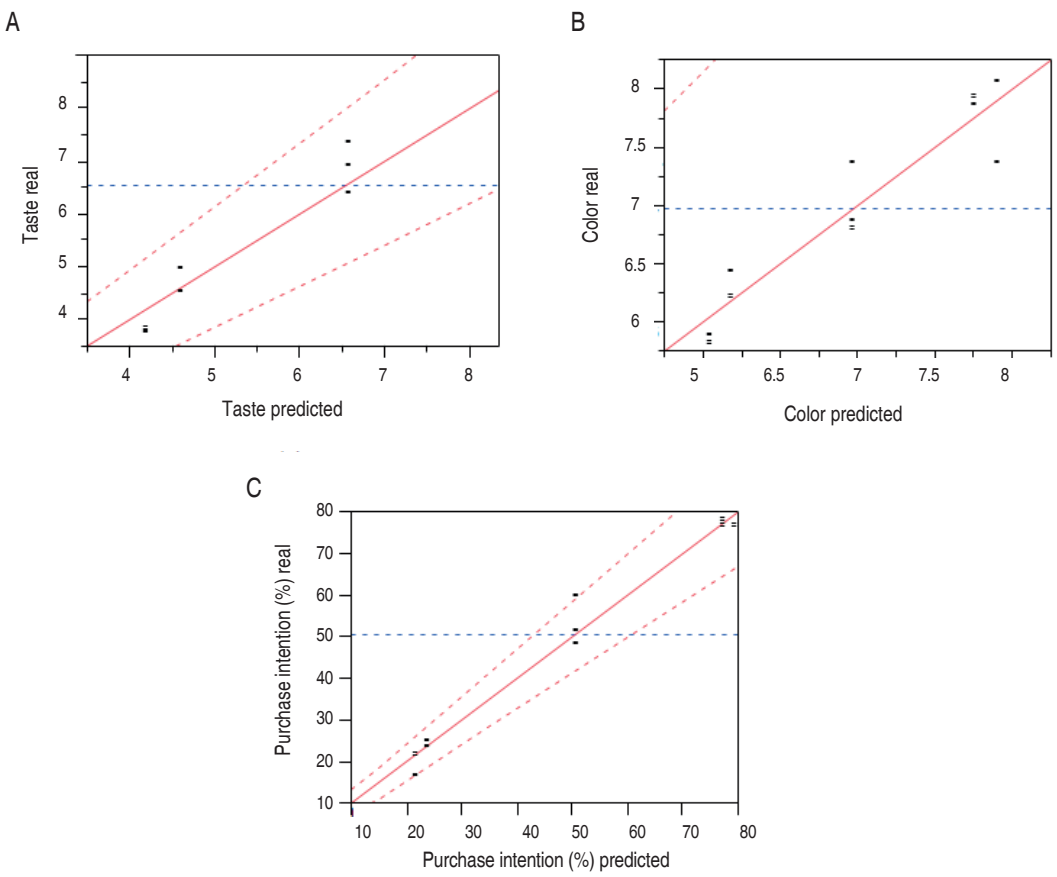

Figure 2. Real vs. predicted values. Variables: A. taste; B. color; C. intention of purchase.

Table 4. Prediction models of the variables.

Variable and Prediction Model		Figure 3	*P*	R^2	RMSE
Taste	$4.589\left[\frac{pulp-0.79}{0.21}\right]+9.137\left(\frac{sugar}{0.21}\right)$	A	<.0001	0.96	0.4532
Color	$6.179\left[\frac{pulp-0.79}{0.21}\right]+7.984\left(\frac{sugar}{0.21}\right)$	B	<.0001	0.90	0.2923
Intention of purchase	$23.787\left[\frac{pulp-0.79}{0.21}\right]+81.976\left(\frac{sugar}{0.21}\right)$	C	<.0001	0.98	4.163

The chosen soursop leathers, to evaluate their characteristics over time (pulp:sugar:citric acid)% WB, were: T_{II} (80:20:0) the optimum mixture, and T_V (89.5:10:0.5) the mid-point; also considered a good selection, from the nutritional point of view, because it has a lower sugar content, the lowest concentration of citric acid, and also has occupied the third place in the acceptability ranking (IV> II> V> I > III). Treatment T_{IV} was ruled out of this part of the study because the addition of citric acid had no significant effects.

Assessment of physicochemical characteristics

Total soluble solids. At zero-day, both leathers T_{II} and T_V had 70 and 64 °Brix, respectively (Table 5), surpassing the minimum of 55 °Brix required for optimum pectic gelification (Damodaran *et al*., 2010). TSS decreased over time for both treatments, a behavior that suggests a gain of water due to a humid environment (31-66% relative humidity). T_{II} (20% sugar) showed significantly ($P \leq 0.05$) higher soluble solids than T_V (10% sugar), from day zero to the end of the evaluation (day 45). It is possible that the high sugar content of T_{II} had influences over the total measure of TSS. Utomo *et al.* (2014) prepared fruit leathers enriched with katuk leaves puree, with a similar soursop pulp content (80-90%) and at constant sugar content (20%), having 52.29 and 56.36 °Brix, respectively. This difference can be attributed to the global mixture composition. In this regard, besides the main ingredients, the leathers were also prepared by the addition of water and Arabic gum. The latter may have caused a dilution effect of the TSS of the mixture.

pH and titratable acidity. At the beginning of the evaluations, the pH was 3.36 and 2.98 for T_{II} and T_V, respectively, favoring pectic gelification during leather formation (Damodaran *et al*., 2010). Meanwhile, the titratable acidity was 15 and 36 g per 100 g fruit leather, respectively; this may occur because of the latest treatment has citric acid. The pH was significantly ($P \leq 0.05$) lower in T_V from day 0 to day 45; accordingly, at minor pH higher acidity, that is why the titratable acidity was significantly higher ($P \leq 0.05$) in T_V from day 0 to day 45. In soursop-katuk leaves puree, leathers the titratable acidity was between 31-44 g per 100 g fruit leather, corresponding to the katuk leaves puree content (Utomo *et al*., 2014).

Ascorbic acid. In this study, the ascorbic acid did not show significant differences between treatments. It seems that the presence of citric acid in the mixture did not promote an additive effect in the ascorbic acid quantification. In soursop-katuk leaves puree leathers, the ascorbic acid was between 78-138 mg per 100 g fruit leather; values greater than those in this study, and increasing with the katuk leaves content, a rich source of ascorbic acid (Utomo *et al*., 2014). It can be seen in Table 5 that the ascorbic acid content decreased over time in both treatments, diminishing 18.2 (T_{II}) and 34.6%, (T_V). Although T_V was prepared through adding citric acid, the preservative effect of this acid did not prevent the loss of ascorbic acid in the soursop leather (Muñoz-Villa *et al*., 2014). Another factor that could promote the loss of Vitamin C is believed to be the type of packaging (transparent cellophane bags), that does

not prevent oxidation losses (Anju *et al.*, 2014). Similar results were found in guava bar (Khan M *et al.*, 2014) and in peach-soy fruit leather (Anju *et al.*, 2014) after 90 days and two months of storage, respectively.

Total polyphenols. It is reported that *Annona* fruits, as raw material, are a source of phenolic compounds. For example, *Annona muricata* L. has 624.2-941.4 mgGAE mg^{-1} pulp (Vit *et al.*, 2014) and *Annona squamosa* 583.45 µg catequina mL^{-1} (Melo *et al.*, 2008) what makes this fruit an attractive alternative to prepare this kind of products. At day 0, the total polyphenols were 355 (T_{II}) and 366 (T_V) mgGAE mg^{-1} soursop leather. It is observed that the lower content in the leathers, compared to the raw pulp (Vit *et al.*, 2014), it is because of the fruit processing and heat treatment during the leather preparation (Hernández *et al.*, 2012). However, it is considered that this total polyphenol content, together with the natural ascorbic acid of the soursop pulp, gives this product healthy characteristics. In soursop leathers, the total polyphenol content decreased over time in both treatments. T_{II} showed significantly ($P \leq 0.05$) lower total polyphenol content than T_V from day 0 to day 45. It is presumed that a higher pulp content gives a higher concentration of total polyphenols in the leather; observable fact when comparing both treatments. On the other hand, the decrease in total polyphenols in the leather is attributable to the type of packaging (transparent cellophane bags) and the storage conditions of this study.

When the physicochemical properties of both treatments were compared (T_{II} and T_V) between days 0 and 45, it was observed that T_{II} is more stable within the 45 days of the evaluations of the soursop leathers.

Table 5. Physicochemical characteristics of the soursop leather.

	Time (d)			
	Total soluble solids (°Brix)			
Treatments	**0**	**15**	**30**	**45**
T_{II}	70.00±2.00	63.00±3.46	58.17±1.04	50.83±0.29
T_V	63.67±0.58	51.33±1.15	45.67±0.76	42.67±0.58
	pH			
T_{II}	3.36±0.04	3.36±0.03	3.36±0.04	3.33±0.01
T_V	2.98±0.02	3.05±0.01	2.94±0.01	2.95±0.02
	Titratable acidity (g malic acid per 100 g soursop leather)			
T_{II}	14.88±0.13	14.25±0.50	12.87±0.07	12.94±0.07
T_V	35.82±0.39	32.75±0.10	31.31±0.34	26.35±0.34
	Ascorbic acid (mg ascorbic acid per 100 g soursop leather)*			
T_{II}	6.54±0.54 a	6.52±0.94 a	6.10±0.95 a	5.35±1.22 a
T_V	6.33±0.54 a	6.17±0.62 a	5.44±0.36 a	4.14±0.93 a
	Total polyphenols (mgGAE per 100 mg soursop leather)			
T_{II}	355.38±2.27	354.07±0.01	351.45±2.27	346.21±0.01
T_V	365.86±0.01	360.62±6.00	359.31±0.01	354.07±0.01

* No statistically-significant differences were found between treatments.

Color analysis. Chroma value, hue angle, browning index (BI) and total color difference (ΔE) are very important color parameters during the fruit leather storage. These were estimated from the experimental data by using Equation (1) to Equation (4), and the results are shown in Table 6.

Chroma values and hue angle indicate that the product is opaque and exhibits a tendency toward yellow. Utomo *et al.* (2014) expressed that the soursop pulp gives the product a yellowish-white color, so that if the amount of the soursop is increased, then the color intensifies. When T_{II} and T_V were compared, T_{II} is opaquer. It is suggested that the differences between both treatments since T_{II} contains more sugar. However, since T_V also contains citric acid, that probably preserves some vividness in the product. In general, chroma tended to increase with time, whereas the hue decreased. This last parameter remained below 90°, in the range of yellow color, with a tendency towards orange over time. Table 6 also shows that the treatment and time do not have a significant effect on the hue angle of the soursop leather storage at room temperature, during the time considered in this study.

Table 6. Color characteristics of the soursop leather.

	Time (d)			
	Chroma value			
Treatments	**0**	**15**	**30**	**45**
T_{II}	16.81±2.93	19.93±0.91	20.32±0.78	21.87±0.93
T_V	13.52±2.72	16.43±2.00	14.45±1.27	16.68±1.29
	Hue angle*			
T_{II}	80.88±4.05 a	78.80±1.99 a	77.16±1.46 a	76.46±2.15 a
T_V	84.21±4.42 a	79.03±1.75 a	78.72±1.79 a	75.42±3.14 a
	Browning index (BI)			
T_{II}	51.58±7.80	61.06±3.67	66.39±3.01	69.51±5.44
T_V	37.62±10.66	52.54±8.20	47.62±5.52	56.86±8.11
	Total color difference (ΔE)			
T_{II}	------	8.98±1.02	8.36±0.98	10.69±0.73
T_V	------	5.22±1.32	5.86±0.35	6.52±1.83

* No statistically-significant differences were found between treatments.

Meanwhile, BI represents the purity of brown color; it is considered as an important parameter associated with browning (López *et al*., 1998; Maskan, 2001). The total color difference (ΔE), which is a combination of L*, a*, and b* -values, is a colorimetric parameter extensively used to characterize the variation of color depending on processing or storage conditions (Maskan, 2001; Mohammadi *et al*., 2008). In the soursop leathers, BI increased, by mean, 5.9 and 9.7 units each 15 days in the soursop leathers T_{II} and T_V, respectively, or a variation of 0.3 and 0.6 units per day. Torres *et al.* (2015), in apple and quince leathers enriched with maqui, found that BI increased 0.1 and 0.3 units per day in apple leathers with and without maqui. The latter values were lower than our results, probably due to the antioxidant effect of the maqui. It is observed that over time the soursop leather darkened corroborating with hue variation. It is suggested that this browning may be due to oxidation reactions of the sugar-containing formulations (non-enzymatic) since heat treatment during leather preparation would inactivate the polyphenol oxidase enzyme (Damodaran *et al*., 2010), and because the packaging of the product does not preserve it from the incidence of light. ΔE determines the difference between the samples analyzed without giving information on the direction of the color difference (Méndez-Robles *et al*., 2018). In this study, ΔE values were calculated in relation to the day zero of storage (control soursop leather); a larger ΔE indicates a greater color change from this reference sample (darkening), according to the tendency of hue angle and BI (Table 6).

CONCLUSIONS

From experimental observations and analysis, it can be concluded that highly acceptable fruit leather can be prepared from soursop pulp. Significant changes

in TSS, pH, total acidity, total polyphenol and color (chroma value, browning index and total color difference) were observed in the sour soup leather for 45 days of storage, at room temperature; and in this conditions, the T_{II} (Soursop 80%: Sugar 20%: Citric acid 0%) resulted more stable between the selected treatments. Finally, in addition to the sensory quality, the content of ascorbic acid and total polyphenols make the soursop leather a product of high nutritional quality, and with potentially healthy characteristics.

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Vermicomposting: a transformation alternative for rumen content generated in slaughterhouses

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Vermicompostaje: una alternativa de transformación del contenido ruminal generado en mataderos

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ABSTRACT

Rumen content is a waste produced in slaughterhouses. This type of solid waste can cause bad odor and atmospheric pollution if discharged directly into the environment. Additionally, it may spread disease due to the nesting vectors, and the resulting leachate can lead to groundwater contamination. The objective of this study was to determine the suitability of rumen content, waste generated in the slaughterhouse of Villapinzón (Cundinamarca), as raw material for biological transformation into vermicompost at the Fertisoluciones facilities. The characteristics of the rumen content were analyzed, and during the transformation process, three volumetric capacities (T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3) of compost bed were evaluated for 105 days. Through a principal component analysis, the data was reduced in two dimensions that explained 65.8% of the total variance; the first component related to the number of juvenile individuals, high pH, low moisture and temperature; and the second component related to numbers of adults individuals and high temperatures. The earthworm growth rate was determined by the food quality, as well as by bed size. Microbiological and physicochemical analyses were performed on the resulting vermicompost, demonstrating that the transformation process of rumen material into organic fertilizer, performed in Fertisoluciones facilities, fulfills the parameters required by the NTC5167 standard. This study showing the possibility of using a slaughterhouse's solid waste and convert it into a valuable product to an industrial scale.

Keywords:
Rumen content
Solid waste
Vermicompost

RESUMEN

El contenido rumen es un subproducto producido en los mataderos. Este tipo de desecho sólido puede causar mal olor y contaminación atmosférica si se descarga directamente al medio ambiente. Además, puede propagar enfermedades debido a los vectores que puede albergar, y el lixiviado resultante puede conducir a la contaminación de las aguas subterráneas. Este estudio tuvo como objetivo determinar la idoneidad del material rumen, residuo generado en la planta de sacrificio de Villapinzón (Cundinamarca), como materia prima para la transformación biológica en vermicompost en la planta de Fertisoluciones. Se analizaron las características del contenido rumen en el proceso de transformación, y se evaluaron tres tratamientos diferenciados por el volumen de las camas de compostaje (T1: 5,94 m^3, T2: 23,01 m^3 y T3: 16,74 m^3), durante un periodo de 105 días. El pH, la humedad, la temperatura y la densidad poblacional de la lombriz fueron monitoreados. A través de un análisis de componentes principales, se redujo la dimensionalidad de los datos a dos dimensiones que explican cerca del 65,8% de la varianza, la primera relacionada con el número de individuos juveniles, la baja humedad y temperatura, y la segunda relacionada con el número de individuos adultos y temperaturas más altas. La tasa de crecimiento de la lombriz estuvo determinada por la calidad del alimento así como por el tamaño de la cama. El vermicompost obtenido se analizó microbiológica y fisicoquímicamente, demostrando que el proceso de transformación de material ruminal en abono orgánico realizado en la planta Fertisoluciones, está dentro de los parámetros de inocuidad exigidos por la NTC 5167. Este estudio muestra la posibilidad de utilizar un residuo sólido de matadero y convertirlo en un producto valioso a escala industrial.

Palabras clave:
Contenido ruminal
Residuos sólidos
Vermicompost

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Solid waste generation in beef cattle slaughterhouses causes sources of contamination. This waste increases the organic load in nearby effluents since it is deposited into water sources or even landfills – a practice that promotes soil, air, and water pollution.

This situation is especially difficult since, in Colombia, 104 of the 286 animal benefit plants have been closed, 179 are open with definitive sanitary authorizations, and two have not been monitored by guvernamental entities yet (Triana, 2019), indicating a more considerable lack of regulation by state entities.

During the first trimester of 2010, approximately 30,000 t of rumen content was produced in Colombia, a high contaminating organic load (Ríos and Ramírez, 2012). The Chemical Oxygen Demand (COD) of slaughterhouse-generated waste is evident from their fats, proteins, salts, and suspended and dissolved solids. It is estimated that one beef cow generates approximately 4.42 kg of Biological Oxygen Demand (BOD_5), 12.64 kg COD, and 4.08 kg of Total Suspended Solids (TSS) (Corantioquia, 2016).

However, rumen content, given its physicochemical and microbiological characteristics, constitutes the ideal raw material for annelid feed and vermicompost production. The decomposition of this material occurs through microbial interactions generated by the presence of polymers, such as cellulose, hemicellulose, lignin, and structural proteins, among other molecules. According to Jara *et al.* (2016), rumen content contains a significant microbial load, which interacts with those microorganisms present in earthworms' digestive tracts (Brito-Vega and Espinosa-Victoria, 2009).

Pseudomonas spp. are microorganisms that inhabit the rumen content (Castro *et al.*, 2018) and play an important role as phosphorus solubilizers (Pincay *et al.*, 2014). On the other hand, the presence of *Aeromona*s sp. (40%), *Bacillus* sp. (37%), *Photobacterium* sp. (10%), *Pseudomonas* spp. (7%), and *Shewanella* sp. (6%) were reported in the digestive tract of earthworms in composted cattle manure at 20 °C, with 70% of moisture (Hong *et al.*, 2011).

Rumen content management with earthworms aids in the stabilization of soil properties, enhances microorganism populations. Plant nutrition processes are also benefited by improving the fertilizers used. Adding organic material to the soil increases the cation-exchange capacity, elevates the water holding content, and simultaneously decreases the apparent density, which increases porosity (Zapata and Osorio, 2013; Blasco and Burbano, 2015).

Rumen content could be a valuable raw material for vermicompost preparation. According to Rafaelli *et al.* (2005), it is composed of a protein percentage of 10.40%, ether extract of 2.84%, a raw fiber of 34.29%, a nitrogen-free extract of 37.21%, ash of 15.85%, neutral detergent fiber (NDF) of 65.14%, acid detergent fiber (ADF) of 41.19%, hemicellulose 23.95% of lignin 14.13%, and cellulose of 27.05%. The aim of this research was to evaluate the quality, in terms of chemical, microbiological composition, physical condition, and enzymatic activity of organic fertilizer obtained from vermicomposted rumen content.

MATERIALS AND METHODS

Geographical location

This study was carried out in the Fertisoluciones S.A. processing plant, located in the department of Boyacá, in the municipality of Ventaquemada, Bojirque settlement in a 6,400 m^2 area at an altitude of 2,829 m, 05°23'46" N, 73°29'00" W, with an average temperature of 11 °C.

Raw material characterization

The rumen content was obtained from the slaughterhouse of Villapinzón municipality (Cundinamarca) and was stored in 10 m^3 piles for 120 days; the piles were covered with a plastic film to prevent contamination and dehydration. The physicochemical analyses were performed according to NTC 5167 (ICONTEC, 2011): Percentage of total oxidizable organic carbon, density, moisture content, percentage of total organic nitrogen, pH, C/N ratio, *Pseudomonas* spp. (CFU count was performed). Phosphatase activity was measured according to the method described by Anderson *et al.* (1975), using p-nitrophenyl phosphate as a substrate for the reaction, which was determined spectrophotometrically at 400 nm. Cellulase activity was determined through the method of Schinner and von Mersi (1990) using carboxymethyl cellulose as substrate; cellulase activity was measured at 540 nm. Both enzymatic activities were measured through a GENESYS TM 20 spectrophotometer and expressed as µmol min^{-1} g^{-1} of p-nitrophenol and glucose, respectability.

Transformation process and physicochemical analysis
The rumen content was piled into beds of three different volumes, corresponding to the experimental treatments: Treatment 1 (T1): 5.94 m^3, Treatment 2 (T2): 23.01 m^3, and Treatment 3 (T3): 16.74 m^3. These were monitored every 15 days for a total of a 105-day evaluation period, i.e., seven sampling times (S1: 15 d, S2: 30 d, S3: 45 d, S4: 60 d, S5: 75 d, S6: 90 d, and S7: 105 d). There were placed a Halthen thermometer in three different locations within the bed piles at a depth between 5 and 10 cm. From each vermicompost bed, 23 samples were taken randomly following a zig-zag pattern, at a depth of 5 and 10 cm, which were mixed to obtain a composite sample of 1 kg. Then, it was separated into two 500-g samples. The first one was used to determine physicochemical characteristics, and the second one was used to determine the density of earthworm populational. The earthworm population density was calculated considering the capsule, juvenile, and adult stages (Equation 1) (Schuldt *et al.*, 1998).

$$earthworms / bed = \frac{earthworms \times bed\ volume(m^3)}{Volume\ of\ sample\ extracted(m^3)} \quad (1)$$

Vermicompost analysis
After 105 days, a sample of 500 g was taken to assess the final product qualities. Physicochemical characteristics, which included: color, particle size, pH, moisture, macronutrients (N, P, K, Ca, Mg), micronutrients (Mn, Zn, Na), Organic Carbon (OC), Cation Exchange Capacity (CEC), the CEC/OC ratio, Electric Conductivity (EC), Water Holding Capacity (WHC), and the C/N density and ratio were evaluated. A sample of 500 g was taken to evaluate the microbiological quality and physic-chemical conditions of the vermicompost according to the NTC5167 standard (ICONTEC, 2011). Enterobacteria, *Salmonella* sp., mesophiles, thermophiles, molds, yeasts, nematodes, protozoa, and *Pseudomonas* spp. were quantified. Additionally, phytotoxic and respirometric tests were performed, and phosphatase and cellulase activity were determined. The results of the final product were contrasted with the NTC5167 standard (ICONTEC, 2011), as well as other vermicomposts which have resulted from similar processes.

Statistical analysis
The data from Vermicompost analyses were analyzed through principal component analysis (PCA) to determine variable correlations and reduce dimensionality (Lê *et al.*, 2008) (R Core Team, 2018). The number of adults, juvenile, and capsule individuals was employed as active variables, while days and treatments were used as supplementary qualitative variables. The Principal components or Dimensions were retained according to Kaiser's rule (Kaiser, 1991).

RESULTS AND DISCUSSION

Raw material quality
Table 1 shows that the OC content is quite high. This value is due to the organic composition of this material, which is composed of carbohydrates (52%), lignin (20%), nitrogenated compounds (12%), lipids (3.5%), organic acids (2.5%), and other components (10%) (Blasco and Burbano, 2015). Thus, this is a complete source of nutrients required for eartworms' metabolism (Espinosa-Victoria *et al.*, 2018). High C content slows decomposition, which may explain the mineralization rate value (Table 1). Khwairakpam and Kalamdhad (2011) reported values of 44.8, 41.1, and 38.4% (on 15, 30, and 45 days, respectively) in the total C content in a mixture of plant residues and vermicomposted cattle manure, it is possibly due to a decrease in carbon content related to earthworms activity, such as, organic matter oxidation; the OC value (41.1%) is similar to that reported here.

In the rumen-based vermicompost, the total nitrogen content was 2.26% (Table 1), higher than vermicomposted materials based on manures from rabbit (1.41%), horse (1.51%), and chicken (1.34%) (Rotondo *et al.*, 2009). These authors mention that N is in mineral and organic form, and its content in the vermicomposting process tends to decrease due to the nitrogen mineralization mediated by earthworms actions. This transformation may be related to the source of food for the cows from which ruminal material was obtained, or to the greater ingestion of tender forage with high content of structural proteins, which is not easily degradated and generates an

increase in nitrogen percentage, following what Eulloque Guerrero (2013) stated. The increase in total nitrogen content is produced by mineralization and microbial fixing processes of atmospheric nitrogen (Camiletti, 2016). Simultaneously, the high organic carbon percentage of the ruminal material is a source of energy for microorganisms, and the nitrogen content contributes to the formation of new microbial cells (Blasco and Burbano, 2015).

Regarding density, it may be affirmed that vermicompost is a very light material. However, it has a high WHC, principally of molecules such as cellulose, hemicellulose, protein, and lignin, which are insoluble in water and permits an increase in weight. It may retain up to 83.2%, as determined in the analysis. This property is in agreement with those reported by Eulloque Guerrero (2013), who found that ruminal content has a moisture average near to 85%. The pH thereof is strongly alkaline, possibly owing to the presence of more anions than cations, similar to Shrestha's *et al.* (2011) findings in a rumen content composted for three months. Methanogenic microorganisms also help to maintain the pH between 7.8 and 8.2 due to their metabolic processes.

Table 1. Chemical, microbiological composition, and enzymatic activity of rumen content at the Villapinzón plant (Cundinamarca).

Parameter	Value
Oxidizable organic carbon (%)	45
Density (g cm^{-3})	0.1
Moisture (%)	83.2
Total nitrogen (%)	2.26
pH	8.18
C/N	19.9
Pseudomonas spp. (CFU g^{-1})	9.5×10^4
Phosphatase activity (µmol min^{-1} g^{-1})	2165
Cellulase activity (µmol min^{-1} g^{-1})	2314

Rumen content reported 9.55×10^4 CFU g^{-1} of *Pseudomonas* spp., within which phosphate and cellulolytic solubilizers may be present. Table 1 also reports enzymatic activity for phosphate and cellulose. The presence of these organisms is a result of phosphate compounds, such as phytin, which are found in grain straw, in rumen content. Also, ATP and phospholipid molecules are affected by phosphate solubilizers. Since cellulose activity was found in the raw material, it could indicate the presence of microorganisms able to synthetize this enzyme, which coincides with the results obtained by Quintero (2014).

pH, moisture and temperature behavior during the transformation

Principal components (PC) or dimensions 1 and 2 were chosen according to Kaiser's rule, which states that PCs with eigenvalues greater than one should be retained (Kaiser, 1991). Dimensions 1 and 2 explain 41.25% and 24.51% of the variance, respectively. In Dimension 1, those with the highest weight were: juveniles, capsules, pH, and the moisture percentage. For Dimension 2, these were temperature and adults (Figure 1).

The influence of temperature and pH on the number of adult individuals is null, which may indicate independence between those variables within the observed values. The number of juveniles tends to be higher in alkaline pH and is not affected by humidity or temperature. Moist, warm environments and acidic pH tend to increase the number of capsules (Figure 1).

Prasanna (2016) reports that, at a temperature range of 0-40 °C, the earthworms perform the vermicomposting process. However, their regeneration capacity is between 25 and 40 °C. Ramnarain *et al.* (2019) showed that an average temperature of 27 °C, similar to the present study. Camiletti (2016) also showed that the vermicomposting process is performed on an industrial scale, as in this case, temperatures may increase up to 35 °C (this increase is controlled with the addition of water), as occurred on day 15 for T2: 23.01 m^3 and day 45 for T3: 16.74 m^3.

On the other hand, bed size does not affect the temperature or pH value thereof, which may occur due to material uniformity. However, the pH behavior may be related to nitrogen transformation in nitrites and nitrates, as well as to transformation reactions of organic phosphorous into orthophosphate (Camiletti, 2016).

Simultaneously, the moisture percentage trend reported an average of 73%, which demonstrates that the transformation process was carried out in optimal moisture conditions. This average coincides with that reported by Eulloque Guerrero (2013), who found that the ideal moisture range for vermicompost was between 60 and 90%.

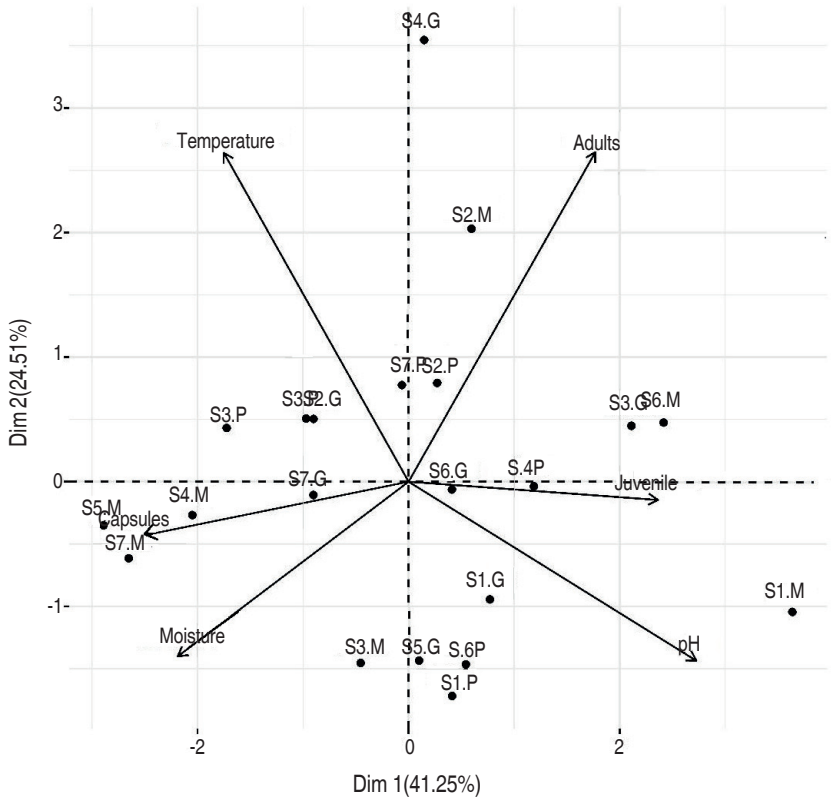

Figure 1. PCA of the different volume treatments (T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3) at seven sampling times (S1: 15 d, S2: 30 d, S3: 45 d, S4: 60 d, S5: 75 d, S6: 90 d, and S7: 105 d) of the vermicompost transformed from ruminal content. The measured variables were the earthworms stages (Capsule, Juvenile, Adult), Temperature, Moisture, and pH of the vermicompost.

Figure 2A shows that for the two dimensions, D1 and D2, extracted in day 15, the number of juveniles in alkaline pH conditions, with low moisture and low temperature, was the greatest. On day 30, the number of adults and temperature increased. On day 45, there was not a detectable treatment net effect. On day 60, something similar to the day 30 occurred, and on day 75, the number of capsules and moisture percentages were high, while the number of individuals was low, the pH was acidic, and the temperature was low. On day 95, the behavior was similar to that of day 15. Lastly, on day 105, it was similar to that of day 75 (Figure 2B). It was observed that there are no differences between the three treatments in terms of the variables studied.

Earthworms' dynamics in a substrate varies, depending on their diet. In this research, it was observed that on day 15, the number of juvenile earthworms was the greatest, which could indicate that the proteins, amino acids, fats, and fiber in the ruminal content satisfy the nutritional requirements for the recently-hatched earthworms eggs. In the ruminal content, the C:N ratio was 19.9, corresponding to carbohydrates and organic carbon compounds (45%), and nitrogenated compounds (2.26% of total N). These contents allowed their development, as observed in the test. Besides, the organic residues ejected by earthworms increase the calcium content in the substrate, for which the pH increased.

Adult numbers increased on day 30, as these correspond to the juveniles that grew from the planted earthworms. According to Bravo *et al.* (2018), each young earthworm that matures may be in reproductive age between 60 and 90 days, or day 30 in this case. This population had the referenced age on average. The same occurred on day 45, when the adult population, which hatched from the cocoons in the substrate. The eggs hatch between 12 and 21 days after oviposition, and each juvenile earthworm matured until reproductive age in approximately 60-90 days.

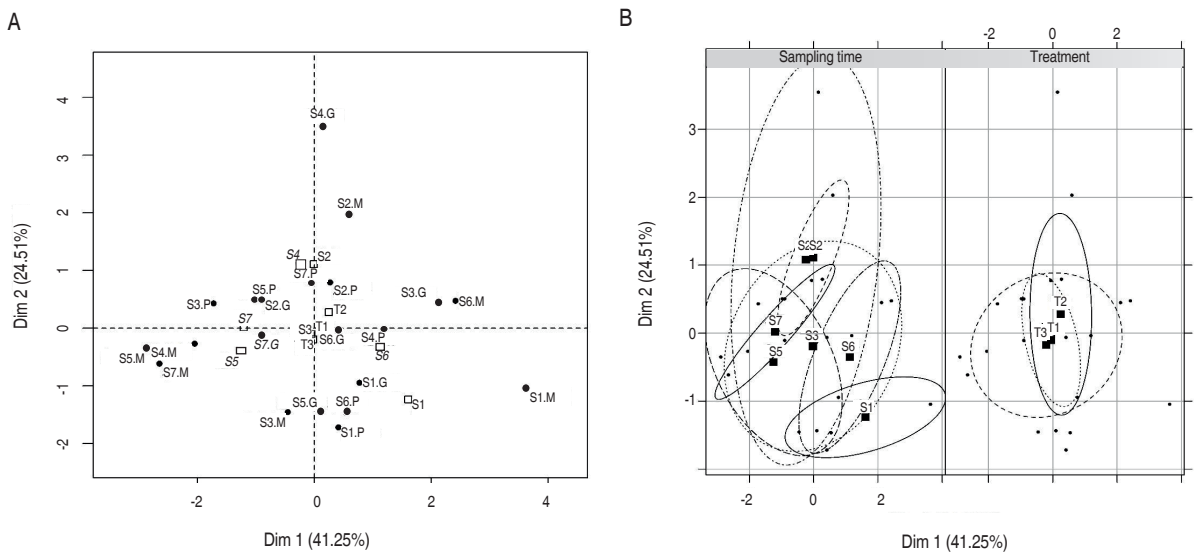

Figure 2. Behavior of individuals corresponding to the mean values of each treatment at different sampling times in the PCA. A. Biplot generated from vermicompost data from three different volume treatments (T1: 5.94 m^3, T2: 23.01 m3, and T3: 16.74 m^3) and seven sampling times (S1: 15 d, S2: 30 d, S3: 45 d, S4: 60 d, S5: 75 d, S6: 90 d and S7: 107 d); B. 95% confidence ellipses surrounding Treatment and Sampling time.

For this reason, it makes sense that on day 75, the number of cocoons or the earthworms which had become adults increased. On the other hand, the number of adults and juveniles decreased because these migrated downwards within the substrate, searching for resources, as the eggs were placed in the processed substrate, which is feed for earthworms recently emerged from their cocoons.

Figure 3 indicates the relationship between the number of individuals per bed for the three treatments. The values obtained correspond to the earthworm states capsules, juveniles, and adults. The T2: 23.01 m^3 presented the highest number of individuals, as compared to the other two treatments. These results indicate that the larger the volume utilized, the higher the earthworm development. Besides, in that volume, there is enough available feed and appropriate pH, moisture, and temperature conditions. The values reported for the present study are higher than those found by Schuldt (2005), who discusses a range between 80,000-120,000 individuals per bed, while values above 130,000 for T1: 5.94 m^3 and over 300,000 for T2: 23.01 m^3 and T3: 16.74 m^3 are reported in the present study. Schuldt *et al.* (2005) also report that, when 100,000 individuals per bed are exceeded, the bed must be expanded to favor earthworm

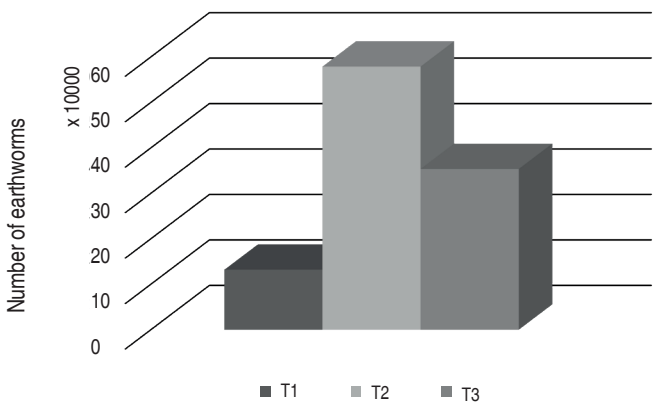

Figure 3. Number of earthworms per treatment after 105 days. T1. 5.94 m^3; T2. 23.01 m^3; T3. 16.74 m^3.

development. The only variable which was influenced by bed size was the number of individuals per bed, as that the larger the volume, the larger the population grew.

Vermicompost characteristics

Physicochemical parameters. The composition of the three treatments for the presence of Ca was higher than fresh manure (0.003%), manure mixed with fruit waste from oil palm in equal parts (0.0022%) (Hernández *et al.*, 2008), and dry cow manure mixed with coffee parchment in equal parts (0.68%) (Contreras *et al.*, 2014). The values obtained for Ca in this study were similar to kitchen waste (1.06%), the coffee pulp (0.84%), and cardboard (0.94%) (Paco *et al.*, 2011). The Mg in waste from the previously-mentioned treatments was 0.38, 0.34, and 0.37%, which is superior to the ones of this study, but for K, it was greatly exceeded by the vermicompost obtained by grape bagasse 1.4%. Cordeiro *et al.* (2013) found the following percentages: for kitchen waste, 1.73%, coffee pulp, 1.74%, and cardboard, 0.91%. These results are related to the vermicompost quality and depend on the raw material fed to the earthworms.

The sum of the Ca, Mg, K, and P percentages were in the range between 3.1-3.5, which indicates that the fertilizer qualifies as an organic amendment, under the NTC 5167 standard (ICONTEC, 2011). Similarly, on the establishment of cationic ratios, it was observed that in the values encountered, there was a deficiency of Mg, Ca, and Zn, which is corroborated by the ash percentage, which was

Table 2. Microbiological parameters of vermicompost analysis in three different volume treatments.

Parameter	Units	T1: 5.94 m^3	T2: 23.01 m^3	T3: 16.74 m^3
Total calcium	%	1.074	1.18	1.147
Total magnesium	%	0.169	0.2074	0.204
Total potassium	%	0.4438	0.557	0.5684
Total sodium	%	0.504	0.66	0.638
Total zinc	%	0.00923	0.0114	0.0088
Cadmium	ppm	ND	ND	ND
Chromium	ppm	≤0.02	6.98	ND
Nickel	ppm	≤0.003	ND	≤0.1
Lead	ppm	≤0.01	ND	≤0.2
Mercury	ppm	ND	0.170	0.13
Arsenic	ppm	≤0.1	1.92	≤0.02
Ash	%	37.2	34.2	38.1
CEC	meq $100g^{-1}$	77.4	77.3	78.7
CEC/CO	meq $100g^{-1}$ CO	313	265	279
Total oxidable organic carbon	%	24.7	29.2	28.2
Electrical conductivity	dS m^{-1}	0.33	0.33	0.34
WHC	%	311	327	314
Density (20 °C)	g cm^{-3}	0.29	0.22	0.2
Total phosphorous	%	1.44	1.52	1.59
Moisture	%	72.9	74	71.6
Total organic nitrogen	%	1.97	2.22	1.88
pH (10%)	-	6.34	6.52	6.23
C/N relationship	-	12.5	13.1	15
Ca/Mg relationship	-	6.35	5.9	5.62
Ca/K relationship	-	2.42	2.11	2.01
Mg/K relationship	-	0.38	0.37	0.35
Ca+Mg/K relationship	-	2.80	2.49	2.37
Particle size >2mm	% dry mass	69.2	76.8	65.3
Particle size	% dry mass	30.8	23.2	34.7

below 40%. The total P reported was 1.44, 1.52, and 1.59% respectively for T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3, which corresponds to the organic and inorganic P. This indicates that the *Pseudomonas* spp. reported in the analyses may be phosphate solubilizers (Awasthi *et al*., 2011), considering the presence of phosphates in the three treatments (Table 2). However, there was a reduction in the activity of this enzyme, which was 2165 µmol min^{-1} g^{-1} in the rumen content, while in the final product, it did not exceed 873.25 µmol min^{-1} g^{-1}. However, this enzymatic activity may favor the availability of phosphorous for the plant, as well as the phosphorous found in the soil, given that it may be activated by the microorganisms added with the fertilizer (Brito-Vega and Espinosa-Victoria, 2009).

The total organic N reflected values of 1.97, 2.22, and 1.88% for T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3, respectively, which are within the parameters established by the NTC 5167 standard (ICONTEC, 2011). It should be noted that the total organic N contained in the initial rumen content was 2.26%, decreased in the T1: 5.94 m^3 and T3: 16.74 m^3 in the final product, and maintained its value in the T2: 23.01 m^3 (Table 1 and 2). However, it is a low value compared to vermicompost obtained from grape bagasse (2.96%) (Cordeiro *et al*., 2013), but superior to the N contained in vermicompost from coffee parchment and manure (0.127%) (Contreras *et al*., 2014), and dry cow manure (0.57%) (Castillo *et al*., 2000). The N quantities herein are slightly superior to those of vermicompost from kitchen wastes (1.25%) (Castillo *et al*., 2000), fresh manure (1.62%), and the 50/50 fresh manure and fruit oil palm waste (1.13%) (Hernández *et al*., 2010), which indicates that wastes of vegetable origin retain more N that remains in the soil after mineralization. It may be affirmed, considering that C/N ratios are high and mineralization rates are low and, that the fertilizer completes the N circle in the soil.

Table 2 demonstrates that the CEC was very high compared to other vermicomposts, such as that of fresh manure and the manure-fruit waste from oil palm, which have 43.72 meq $100g^{-1}$ and 47.24 meq $100g^{-1}$, respectively (Hernández *et al*., 2010), but are slightly above those of coffee parchment-manure (Contreras *et al*., 2014). This behavior implies that this material increases soil fertility when it is applied, by retaining free cations in solution. The increase thereof is variable in rumen content and may owe to the molecules that compound this material (cellulose, lignin, hemicellulose, protein); when breaking down, it produces smaller molecules, such as organic acids, amino acids, or phenols.

Total oxidable organic carbon (OC) values were reported at 24.7, 29.2, and 28.2% for T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3, respectively, which are slightly lower than the NTC 5167 standard (ICONTEC, 2011), and higher than those reported by Paco *et al.* (2011) for kitchen waste (10.85%), the coffee pulp (11.37%), or cardboard (11.49%). Besides, it was lower than vermicompost from grapevine bagasse (34.59%) (Cordeiro *et al*., 2013). The low vermicompost values for rumen content result in a high mineralization rate. Following the values reported, it would classify as a non-humic organic amendment, according to NTC 5167 (ICONTEC, 2011). As a result of these two variables, the CEC/CO indicates that the organic matter percentage, which makes the cation exchange has a relationship over 200 being a high value for this variable.

The C/N ratio, with values of 12.5, 13.1, and 15 for T1: 5.94 m^3, T2: 23.01 m^3, and T3: 16.74 m^3, respectively, were higher than the final product of grape bagasse (12.6) (Cordeiro *et al*., 2013), the mixture of coffee parchment and manure (12.5) (Hernández *et al*., 2010), or pure cow manure (10.76), but lower than that of the mixture of manure with waste fruit from oil palm (19.2) (Contreras *et al*., 2014). Values above 12 indicate that the material has a slow mineralization rate that brings few inorganic nutrients to plants; however, it becomes a source of feed for the organisms in the soil, as the physicochemical conditions change.

None of the treatments reported high CE. In other words, there was no presence of salts. Similarly, compared to other materials, it was quite low: fresh manure (5.38%), palm oil fruit waste-manure (3.46%) (Hernández *et al*., 2010), and coffee parchment -manure (2.32%) (Contreras *et al*., 2014).

The WHC was high with values that reached 311-327-314% (T1: 5.94 m^3-T2: 23.01 m^3-T3: 16.74 m^3), according to the same rumen material composition. This capacity indicates that this material can retain three times more water than its weight, favoring the soil into which it is

applied. Hydrogen bridges retain the water, and negative charges are generated by the organic matter in molecules like amino acids, phenols, and organic acids, among others.

The moisture content of the material was more than 70%, as shown in Table 2 (at the time that samples were taken). This corresponds to the ideal moisture level for earthworm habitats. However, it should be reduced to 30% via outdoor drying, which permits microbial and enzymatic activity and let compliance with the requirements of the NTC 5167 standard (ICONTEC, 2011).

The pH reported in the three treatments indicates that this material is slightly acidic, while Paco *et al.* (2011) tests indicate that the pH of kitchen wastes was 7.79, the coffee pulp was 7.48, classified as alkaline, the vermicompost obtained from cardboard had a pH of 7.03, and grapevine bagasse of 7.1 (Cordeiro *et al*., 2013). These values are neutral or almost neutral. The vermicompost's pH obtained from pure cow manure was measured 6.85, while the 50/50 mixture of manure and palm oil fruit waste was 6.54 (Hernández *et al*., 2010). On the other hand, for the vermicompost created from parchment coffee and cow manure, the pH was found to be 6.6 (Contreras *et al*., 2014). Values below 7.0 were classified as slightly acidic, and maybe a result of the presence of organic acids of low molecular weight.

The presence of heavy metals in Table 2 indicates that all of these were below the ranges required by the NTC 5167 standard (ICONTEC, 2011). A direct relationship was not observed between bed size and microbial burden considering the different treatments evaluated. This likely originated via the non-homogeneous distribution of vermicompost, or in the sample collection process.

Microbiological parameters. Pathogenic microorganisms, such as yeasts, nematodes, and *Salmonella* sp., were not found on a general level. In the case of enterobacteria, the counts obtained from the three treatments indicates the none surpassed the limits established by the NTC 5167 standard (ICONTEC, 2011). This absense is favorable, as high pathogen counts inhibit the growth of microorganisms of beneficial origin, which contribute to the added value of vermicompost. In the T3: 16.74 m^3, a higher count of enterobacteria was observed. This microbial content is related to a higher C/N ratio. These elements are fundamental to the reproduction process of the various microorganisms, as well as enzyme production, which helps to degrade insoluble compounds.

Mesophilic bacteria decrease as bed size increased. This group of microorganisms is used as indicators to evaluate different types of treatments, as they serve to inoculate beneficial microorganisms or reduce pathogenic bacterial load. The data from the present study agree with that obtained by Pérez *et al.* (2008), who worked with vermicompost based on coffee pulp, ground coffee pulp, and sheep manure.

The concentration of *Pseudomonas* spp. in the vermicompost sample was 1.5×10^5 CFU g^{-1} in T1: 5.94 m^3, 2.1×10^5 CFU g^{-1} in T2: 23.01 m^3, and 1.6×10^5 CFU g^{-1} in T3: 16.74 m^3 (Table 3). This microorganism increased significantly, compared to rumen content, which may be related to the contribution of microorganisms from earthworms' digestive tracts. These *Pseudomonas* spp. have been used as promotors of vegetative growth, and various strains can produce oxalic, fumaric, and citric acids, as well as phosphatases, facilitating the solubilization of organic and inorganic phosphorous (Awasthi *et al*., 2011). It also controls pathogenic microorganisms, producing antifungal and antibiotic compounds. Similarly, they play a more competitive part, owing to the nutrients available in the soil, and produce siderophores, organic compounds that capture iron. Besides, some species are considered proteolytic, according to the findings in similar studies carried out by García (2006).

In the case of thermophilic microorganisms, high counts were obtained, even when the vermicompost's productive process did not exceed 30 °C. It is notable, as this microbial group has an optimal growth temperature of 40 °C and beyond, and that applied in the present study was lower.

Moisture is a determining factor for molds because their metabolic processes would be greater moisture environments, which would yield higher numbers. However, for T2: 23.01 m^3, which presented the greatest moisture levels (74%), no significant increase in molds was found. Nevertheless, the T1: 5.94 m^3, which presented moisture levels of 72.9%, presented the highest numbers (6.0×10^3 CFU g^{-1}). It is important to highlight that, in the analyzed samples, there was no presence of yeast. This absence

indicates that anaerobic and alcoholic fermentations do not occur.

On a general level, the pH did not reflect any important variation, which would represent a significant difference in the microorganism counts obtained. The pH was nearly neutral. It should be considered that samples for the analyses were taken before the drying and sifting stages, which may have caused non-homogeneous distribution, and caused certain organic additions and accumulation of nutrients and microorganisms. Generally, these results demonstrate the quality of the product obtained under the NTC 5167 standard's values (ICONTEC, 2011).

As mentioned by Espinosa-Victoria *et al.* (2018) and Brito-Vega and Espinosa-Victoria (2009), the high microbial content in vermicompost is due to earthworms have approximately 500 billion individuals in their intestinal tract, which affects the data obtained in the fertilizer. Additionally, as vermicompost does not have a thermophilic phase, in which, typically, these organisms would be eliminated, more of them are present.

The OC levels reported in Table 3 were measured following a test in which the processes were utterly anaerobic. According to what was reported by Banerjee *et al.* (2018), respiration rates are directly related to the stability of the worm compost, indicating that it is a very stable compost. The three treatments with respiration rates lower than 2 indicate mature vermicompost, which has completed the decomposition stage and does not present a potential phytotoxicity risk.

Table 3 showed that the final product complies with the NTC 5167 standard (ICONTEC, 2011), in terms of the presence of pathogenic microorganisms such as *Salmonella* sp., enterobacteria, nematodes, and protozoa. This happens because the pH is slightly acidic, and the stable product's moisture level is lower than 30%. These conditions are not favorable for said organisms. However, Espinosa-Victoria *et al.* (2018), showing that in earthworms' digestive tracts, there are at least 16 species of *Bacillus* sp., among others, which are ingested by way of the food they consume. It means that rumen content is free of these pathogens and that the earthworm breeding process is not contaminated.

Within the enzymatic biotransformation processes, the presence of the earthworm has been found to stimulate the activity of enzymes (Villegas-Cornelio *et al.*, 2017).

Table 3. Microbiological parameters of vermicompost analysis in three different volume treatments.

Parameter	Units	T1: 5.94 m^3	T2: 23.01 m^3	T3: 16.74 m^3
Mesophiles	CFU g^{-1}	5.80×10^7	1.60×10^7	5.10×10^7
Thermophiles	CFU g^{-1}	1.00×10^6	2.30×10^7	3.70×10^7
Molds	CFU g^{-1}	6.00×10^3	2.00×10^3	1.00×10^3
Yeasts	CFU g^{-1}	0.00	0.00	0.00
Nematodes and/or protozoa	Absence-presence	0	0	0
Enterobacteria	CFU g^{-1}	1.00×10^2	2.00×10^2	9.00×10^2
Salmonella sp.	CFU $25g^{-1}$	0	0	0
Respirometry	mg CO_2 g^{-1}	0.22	0.26	0.26
Pseudomonas spp.	CFU g^{-1}	1.5×10^5	2.1×10^5	1.6×10^5
Phosphatase	µmol min^{-1} g^{-1}	873.25	785.9	570.30
Cellulase	µmol min^{-1} g^{-1}	2703.0	2590.6	3206.10

However, those microorganisms that inhabit their gastrointestinal tract have the enzymatic machinery to perform this activity, and, as observed in the final product result, phosphate activity decreases, compared to the value in the rumen content. This behavior is probably due to earthworms in their growth and development, consume the phosphorous present in the raw material, and this causes a decrease in its enzymatic activity in the final product. In

contrast, for cellulase activity increases because the fiber content is higher when cellulose is present.

In general, it is important to consider that vermicomposting is attracting researchers' attention in recent years owing to the reason that this method can degrade organic wastes and can recycle and convert valuable nutrients into organic fertilizers. The industrial-scale conversion of waste as rumen content presents several challenges; however, this study shows that it can be used to obtain an amendment that complies with the regulatory parameters established in Colombia, leaving open the possibility of exploring potential uses for earthworms which develop correctly on this type of material.

CONCLUSIONS

The rumen content transformed by earthworms into organic fertilizer shows optimal qualities regarding other solid wastes, which may be alternatives for the management of this by-product of agroindustrial processes. Earthworms' growth rates are determined by the quality of the food they ingest, and the population rate is determined by bed size. In this research, a large volume bed for vermicomposting contributes positively to Earthworms' development; it stimulates the generation of individuals. The microbiological and physicochemical analyses performed to demonstrate that the transformation process from ruminal material on a large scale allows getting organic amendment with good quality and satisfy the NTC 5167 standard's parameters.

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Economic injury level for the flower thrips *Frankliniella* cf. *gardeniae* Moulton (Thysanoptera: Thripidae) in mango

Nivel de daño económico de los trips de las flores *Frankliniella* cf. *gardeniae* Moulton (Thysanoptera: Thripidae) en mango

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ABSTRACT

Mango is a tropical fruit with great production and market worldwide. In Colombia, it is an important crop with high export potential. There are many arthropods affecting mango production, such as thrips that feed on leaves, flowers, and small fruits, causing damage such as deformations, scars, and premature fall of fruits. This study aimed to establish the economic injury level (EIL) of *Frankliniella* cf. *gardeniae* Moulton in mango (*Mangifera indica* L. var. *yulima* and var. *tommy*) in Guamo and San Luis (Tolima, Colombia). The methods employed included a weekly sampling of thrips in mango inflorescences during two productive periods (B-2015 and B-2016) using the structure tapping technique in a deep tray with mesh. Three preliminary thresholds per productive period were established: [B-2015: ≥10 thrips per inflorescence (TpI), ≥30 TpI, and ≥60 TpI. B-2016: ≥5 TpI, ≥10 TpI, and ≥15 TpI]. The variables density, costs, and yield were recorded. The EIL was calculated statistically using linear regressions and the final action threshold was estimated. An EIL of 11 TpI and an action threshold of 7 TpI were estimated for var. *tommy* in Guamo in the second semester of 2016. There was a risk of losing 220 kg ha^{-1} for each increase in insect unit in var. *tommy*. Therefore, thrips in mango behaved as an occasional pest, requiring control in var. *tommy* in Guamo, when the crop is flowering, and the population exceeds the action threshold of 7 TpI.

Keywords:
Action threshold
Inflorescence
Pest
Sampling method
Tropical fruits

RESUMEN

A nivel mundial el mango es una fruta tropical de gran importancia por su producción y mercado, teniendo en Colombia un gran potencial de exportación. Existen muchos artrópodos que inciden en la producción del mango, como son los trips, que se alimentan de hojas, flores y frutos pequeños, generando daños como deformaciones, cicatrices y caída prematura de los frutos. Esta investigación tuvo como objetivo determinar el nivel de daño económico (NDE) de *Frankliniella* cf. *gardeniae* Moulton en el cultivo de mango (*Mangifera indica* L. var. *yulima* y var. *tommy*) en el Guamo y San Luis (Tolima, Colombia). La metodología consistió en muestrear semanalmente los trips en inflorescencias durante dos periodos (B.-2015 y B-2016) productivos del cultivo, mediante el método del golpeteo de la estructura en una bandeja honda con malla. Se establecieron tres umbrales preliminares (límites poblacionales) por periodo productivo evaluado [B-2015: ≥10 trips por inflorescencia (TpI), ≥30 TpI, ≥60 TpI. B-2016: ≥5 TpI, ≥10 TpI, ≥15 TpI]. Se registraron las variables densidad, costos y rendimiento. El NDE se calculó estadísticamente mediante regresiones lineales y se estimó el umbral de acción final. Se calculó un NDE para *tommy* (Guamo) en el semestre B-2016 de 11 TpI y un umbral de acción de 7 TpI, existiendo riesgo de perder 220 kg ha^{-1} por cada incremento de unidad del insecto. Por tanto, los trips en mango se comportaron como una plaga ocasional, siendo su manejo necesario para la variedad *tommy* en el Guamo, cuando el cultivo esté en floración y la población sobrepase el umbral de acción de 7 TpI.

Palabras clave:
Umbral de acción
Inflorescencia
Plaga
Método de muestreo
Frutas tropicales

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Basic products, especially tropical fruits, have acquired great importance in the commerce at the global level, showing an annual growth rate of 5.5% in the year 2017, and registering an increase of 1.9% compared to the average of the last ten years. Mango occupies the first place of importance among the four main tropical export fruits (pineapple, papaya, avocado, and mango) since it contributes with about 51% of the total production (Altendorf, 2017).

In Colombia, domestic production in 2017 was 260,300 t registering an increase of 0.47% compared to 2016, and with an average yield of 12.4 t ha^{-1}. Tolima was the second department with the largest participation in mango production in the country in 2017 with 26.63%, but it was the first in yield with an average of 15.8 t ha^{-1} (SIOC, 2018). However, a limitation that mango has, is that worldwide production is mostly consumed internally in the same producing countries because international markets are very demanding in phytosanitary standards and certifications (Altendorf, 2017).

In order to venture into international markets, adequate management, adapted to export standards and profitable for producers, must be implemented. Besides serious quarantine problems such as the fruit fly *Anastrepha obliqua* (Diptera:Tephritidae), there are other emerging pests such as thrips *Frankliniella* spp. (Thysanoptera: Thripidae), which can increase their populations to harmful levels when inefficient agronomic practices are applied – in many cases, farmers confuse the damage of thrips with fungi attacks (Lopes *et al*., 2002). Thrips cause deformations, spots, scars, and in severe attack cases, the premature fall of leaves, inflorescences, and small fruits (Aguirre *et al*., 2013). In mango, the inflorescences and small fruits show considerable damage with great populations of 1,500 thrips per inflorescence, which affect fruit development and formation (Virgen *et al*., 2011).

This pest insect has an accelerated population growth because it has a short life cycle and an early sexual maturity, which makes its control difficult. This pest shows cryptic habits, a small size, and has an easiness to acquire resistance to insecticides (Bacci *et al*., 2008; Nondillo *et al*., 2009; Santos *et al*., 2012). For these reasons, thrips are described as "r" pests or strategists (Santos *et al*., 2012).

Hence, it is of vital importance to know the moment in which thrips populations reach levels that cause economic losses. This is related to two key concepts. The first one is the economic injury level (EIL), which is the lowest population density that causes economic losses (Pedigo and Rice, 2014). The second one is the action threshold (AT), which is the pest population that warrants a control action in order to prevent the reaching of the EIL (French, 1989; Moreno *et al*., 2002; Pedigo and Rice, 2014).

Due to the importance of this pest in mango cultivation in Colombia and in order to carry out integrated management of thrips in this crop, the economic injury level was calculated, and its population fluctuation was established in two localities (Sierra, 2017). The current work was derived from the previous study to establish the economic injury level (EIL) of *Frankliniella* cf. *gardeniae* Moulton thrips in mango (*Mangifera indica* L. var. *yulima* and var. *tommy*) in two municipalities of the department of Tolima.

MATERIALS AND METHODS

Study area

The research was carried out in the municipalities of El Guamo and San Luis, department of Tolima, Colombia at (4°01'41" N, 74°58'12.0" W), and (4°07'56" N, 75°05'44.0" W), respectively. San Luis has an average temperature of 26 °C located at an altitude of 500 masl, and has an annual rainfall of 1,458 mm (Weather Spark, 2019). On the other hand, Guamo is located at an altitude of 326 masl, with an average temperature of 28 °C and an annual rainfall of 1,488 mm. The mango trees assessed in each farm were between 9 to 15 years old and were planted at a density of 156 trees ha^{-1}, in plots of about 60 ha.

Calculation of the economic injury level of thrips

The range of thrips fluctuation in each of the two farms (localities) assessed was established by preliminary sampling. Then, the average ranges, classes, and amplitudes of each preliminary action threshold (PAT) treatment were established (Santos *et al*., 2012). Samplings were conducted in two mango production periods (semester B of 2015, and semester B of 2016). In the two sampling periods, the mango varieties *tommy* and *yulima* were evaluated; these were selected as they were

the ones with the largest planted area in the region and with the highest market trade.

The PAT treatments of semester B-2015 consisted in maintaining the thrips populations under an established value by using insecticides as follows: T1: 10-29 thrips per inflorescence (TpI), T2: 30-59 TpI, T3: >60 TpI, and T4: undefined TpI (control without application of insecticide). On the other hand, in the B-2016 semester, the PAT treatments were the following: T1: 5-9 TpI, T2: 10-14 TpI, T3: >15 TpI and T4: undefined TpI (control without application of insecticide).

The experimental design in both productive periods was a randomized complete blocks design (the blocking factor was the slope). The experimental plot included four trees, and four repetitions per treatment were used for a total of 16 experimental units. The sampling unit included four inflorescences per tree selected at random from the middle and lower part of the tree.

The thrips population sampling was carried out by tapping the inflorescence (3 times) on a white plastic container or tray (5 cm deep) with a mesh on top (in order to optimize the display and counting of individuals). In each sample, 50% of the effective plot (two trees per block) was evaluated with a weekly frequency in the flowering period, which lasted approximately five weeks (Sierra, 2017).

The density of TpI was calculated by dividing the number of thrips found by the number of inflorescences sampled per treatment. When the insect population on average exceeded the maximum density of each treatment, chemical control was carried out – i.e., applying insecticides to the four trees of the plot. The products used were Spinetoram in doses of 1 cc L^{-1} (Exalt® TM 60 sc) and sulfoxaflor with doses of 0.5 cc L^{-1} (Closer®) (DEAQ, 2016). Both active ingredients act through ingestion and contact, attacking the central nervous system of the insect; besides, sulfoxaflor has a translaminar effect on the plant, which causes a higher residual effect (DowAgroSciences, 2018). The calculation of the relationship between the average TpI densities and mango fruit yield (kg ha^{-1}) was made per treatment with Equation 1 to establish the EIL (Nakano *et al*., 1981; Pedigo *et al.,* 1986; Pedigo and Rice, 2014).

$$EIL = \frac{C}{VIDK} \quad (1)$$

Where:
K: Efficiency percentage of the control method used estimated by the Abbott formula comparing the control (without insecticide) with all the treatments (with insecticide).
V: Value per unit of fruit production in the market in Colombian currency (COP kg^{-1}).
C: Handling control cost per production unit (COP kg^{-1}) – i.e., the necessary cost to bring the population to zero individuals (thrips) in inflorescences (Cardona, 1999; Santos *et al*., 2012).
I: Damage per insect unit.
D: Damage function estimated through a linear regression $Y=a+bx$, employing the statistical software SAS (SAS Institute, 2009). Y is the yield per area; a is the intercept constant; b is the yield loss per insect; and x is the number of insects per inflorescence (Pedigo and Rice, 2014).

Subsequently, the action threshold (AT) was established to obtain knowledge on the insect population in which a control action must be taken and prevent the pest from causing economic damage to the crop. The following variables were included: the economic injury level (EIL), and the thrips population growth rate (TPGR) expressed as thrips per inflorescence per day obtained by linear regression of the average thrips per inflorescence per tree of the control treatment, considering days after flowering (time). Another variable considered was the time between sampling (TBS) expressed in days and the efficiency percentage (K) of the control method. The calculation is expressed in Equation 2, implemented by Santos *et al.* (2012).

$$AT = (EIL - (TPGR \times TBS)) \times K \quad (2)$$

RESULTS AND DISCUSSION

Action threshold for thrips in the *yulima* variety

In the first productive cycle evaluated (B-2015) the population densities of TpI were below the PAT in the two municipalities and no statistically significant relationship was found with the yield (Guamo: P=0.3178, RMSE=538.43, R^2=0.46; San Luis: P=0.7307, RMSE=1,754.29, R^2=0.072). On the other hand, the costs of PAT did not show a statistically significant relationship with TpI density (Guamo: P=0.8528, RMSE=53,818.01, R^2=0.02; San Luis: P=0.8207;

RMSE=19,718.92, R^2=0.03) (Figure 1). In Guamo, it was registered PAT ≥10 and ≥60 with 90,721±6,444 and 122,392±5,699 COP ha^{-1}, respectively. In San Luis, it only registered a PAT ≥10 with 46,289±1,086 COP ha^{-1}. Theses results did not allow calculating the AT for the *yulima* variety in San Luis and Guamo in the first production cycle.

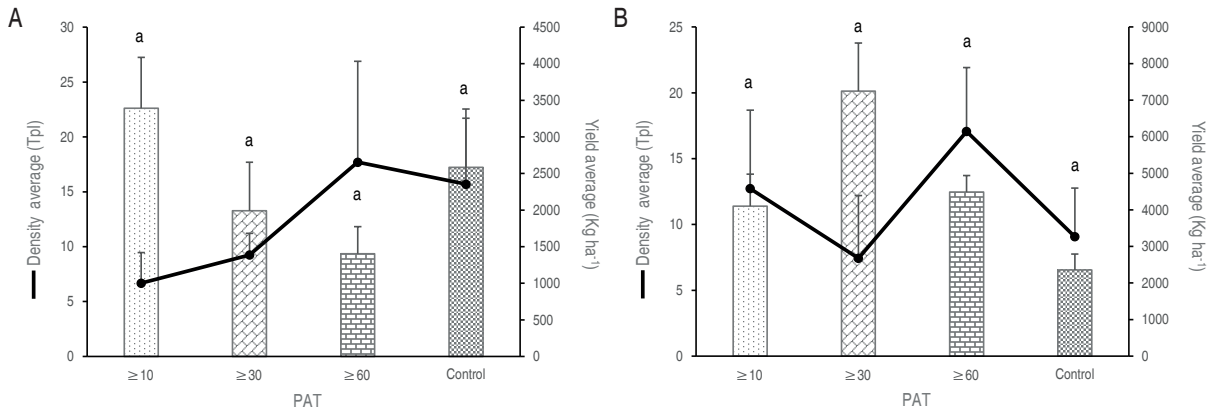

Figure 1. Preliminary Action Thresholds (PAT) for the *Frankliniella* cf. *gardeniae* thrips in mango var. *yulima*, related with density average (TpI) and to the yield (kg ha^{-1}), in the semester B-2015, Tolima. A. Guamo; B San Luis.

Similarly, the AT of var. *yulima* was not estimated in the second productive cycle (2016 B) in the municipalities of Guamo and San Luis because linear regressions between TpI density and yield were not statistically significant (Guamo: P=0.0580, RMSE=4,327.45, R^2=0.21; San Luis: P=0.2880, RMSE=1,031.27, R^2=0.50), the same for TpI density and costs (Guamo: P=0.1035, RMSE=4,601.72, R^2=0.80; San Luis: P=0.2882, RMSE=39,680.1, R^2=0.45) (Figure 2). In San Luis, it was registered 20,755±4,956 COP ha^{-1} for PAT ≥10 and ≥60. In Guamo, the cost was higher in PAT ≥10 with 139,179±39,757 COP ha^{-1}, followed by ≥30 69,589±3,957 COP ha^{-1} and ≥60 20,774±3,957 COP ha^{-1}.

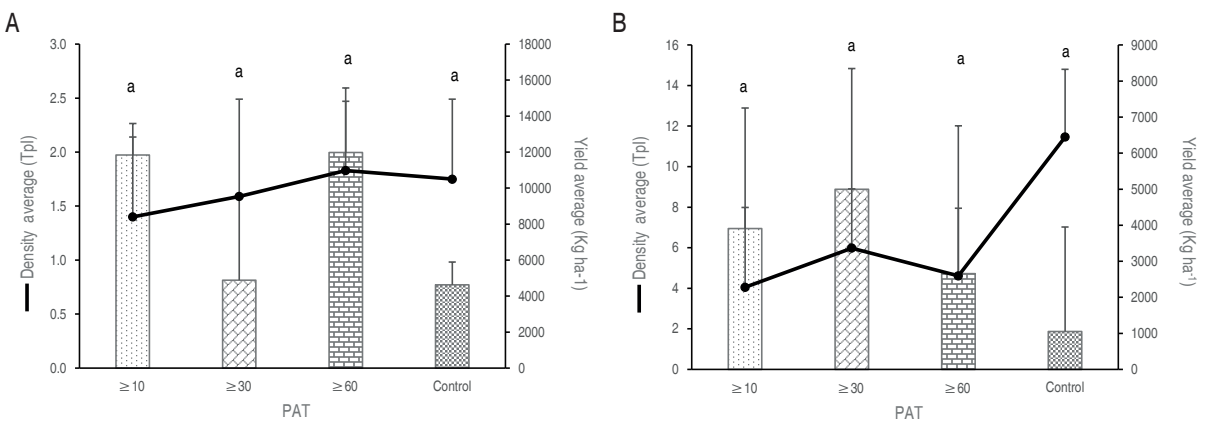

Figure 2. Preliminary action thresholds (PAT) for the *Frankliniella* cf. *gardeniae* thrips in mango var. *yulima*, related with density average (TpI) and to the yield (kg ha^{-1}), in the semester B-2016, Tolima. A. Guamo; B. San Luis.

In the mango crops under study, thrips behaved like occasional pests, which agrees with Durán (2012). Since the optimum of food such as inflorescences and small fruits of 8 mm is exhausted, the population is considerably reduced. Therefore, when the mango crop ends, the phenological stage of flowering and the fruits are larger, the chemical control for this pest becomes unnecessary (García-Escamilla *et al*., 2016). However, in this study, the populations of thrips evaluated during the flowering period of the *yulima* variety did not represent a threat to the

mango crop in the two municipalities evaluated because populations were always below the predefined thresholds. Nevertheless, for thrips to act as a pest in mango, their populations must be high and constant during flowering (Ortiz *et al*., 2016). Mango inflorescences tolerate high losses of flowers because they are comprised of around 3,500 flowers (var. *ataulfo*); for this reason, it withstands mild attacks of anthophila insects without affecting its fertilization (Ortiz *et al*., 2016).

As the range of thrips population observed in this study in the *yulima* variety was not representative. Alternatively, preventive insect control strategies can be implemented, including the use of repellents and trap plants (García-Escamilla *et al*., 2016) as well as the release of biological control agents. This should be done in order to avoid that the insect populations become a pest for mango and cause economic damage.

Since both the climate and the human activities are changing, and besides, thrips show variability in their biology and behavior, these insects can occupy new geographical niches and act as a pest, when conditions are favorable for their population (Morse and Hoddle, 2005). This situation may occur in the future in the municipality of Guamo for the y*ulima* variety. This forecast might be stated because the statistical significance was close to 5% between the density of thrips and the yield.

Action threshold for thrips in the *tommy* variety

The first productive cycle (2015 B) of this variety did not register a statistically significant relationship between the TpI density and the yield in the two municipalities evaluated (Guamo: P=0.4563, RMSE=618.53, R^2=0.29; San Luis: P=0.4373, RMSE=300.61, R^2=0.31). Likewise, this behavior was also found with the TpI density relationship and costs (Guamo: P=0.5558, RMSE=19,240.02, R^2=0.19; San Luis: P=0.757, RMSE=20,292.19, R^2=0.05) (Figure 3). In Guamo, it was registered no costs in all PAT, and in San Luis, costs were reported in PAT ≥5 and ≥10 with 49,595±2,938 and 49,595±2,938 COP ha^{-1}, respectively. Since there was no significant relationship between the evaluated variables, the damage level was not calculated for this production cycle.

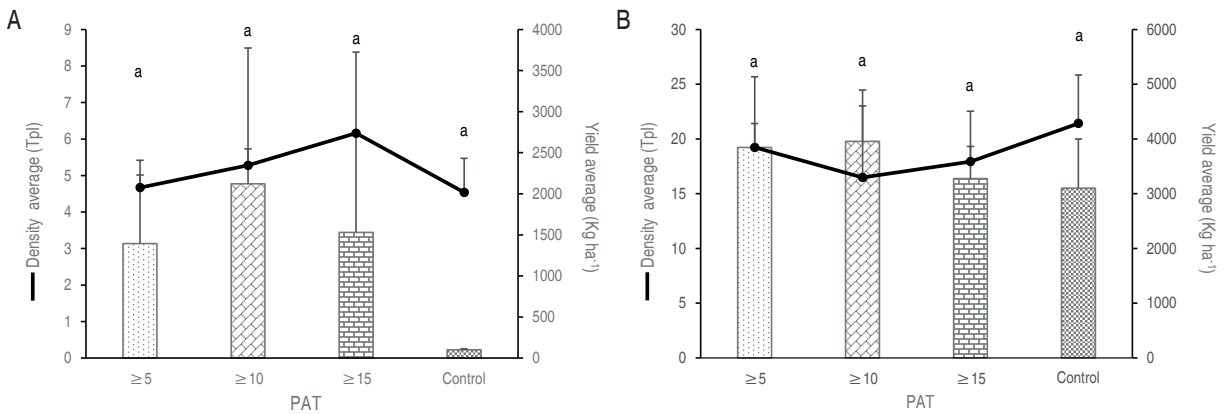

Figure 3. Preliminary action thresholds (PAT) for the *Frankliniella* cf. *gardeniae* thrips in mango var. *tommy*, related with density average (TpI) and to the yield (kg ha^{-1}), in the semester B-2015, Tolima. A. Guamo; B. San Luis.

In the second productive cycle, in the municipality of San Luis, no statistically significant relationship was found between density (TpI) and yield (P=0.2112, RMSE=2,190.91, R^2=0.62) and between density and costs (P=0.8066, RMSE=53,797.46, R^2=0.006); only a PAT ≥5 registered 48,815±24,407 COP ha^{-1}; therefore, AT was not calculated for the *tommy* variety in San Luis (Figure 4B).

Conversely, the *tommy* variety in Guamo showed a statistically significant relationship between density and yield (P=0.0257, RMSE=935.14, R^2=0.35) (Figure 4A), and between density and costs (P=0.0045, RMSE=11,716.46, R^2=0.99), the highest cost was 111,139±20,306 COP ha^{-1} in PAT ≥5, followed by 62,324±20,306 COP ha^{-1} in PAT ≥10 and ≥15. Subsequently, the EIL was calculated in Guamo for the *tommy* variety, as follows:

$$EIL = C/VbK,\ EIL = (1{,}074{,}206)/(1{,}000 - 220.17 - 0.00) = 10.64 \approx 11 \text{ TpI}$$

The variables of the equation above were estimated by recording the average price of mango, which was 1,000 COP kg^{-1} (V) (Rincon *et al.,* 2019). On the other hand, the effectiveness of the insecticides (K) used (spinetoram and sulfoxaflor) was considered as being 80% according to records stated by the Colombian Ministry of Agriculture (MADR, 2020). The management cost per production unit (C) generated by the applications of the insecticides concerning the TpI density was adjusted to a simple linear regression, as follows.

$$Y = 6.56 - 3.5 \times 10^{-6} x \quad (P = 0.0045,\ \text{RMSE} = 11{,}716.46,\ R^2 = 0.99)$$

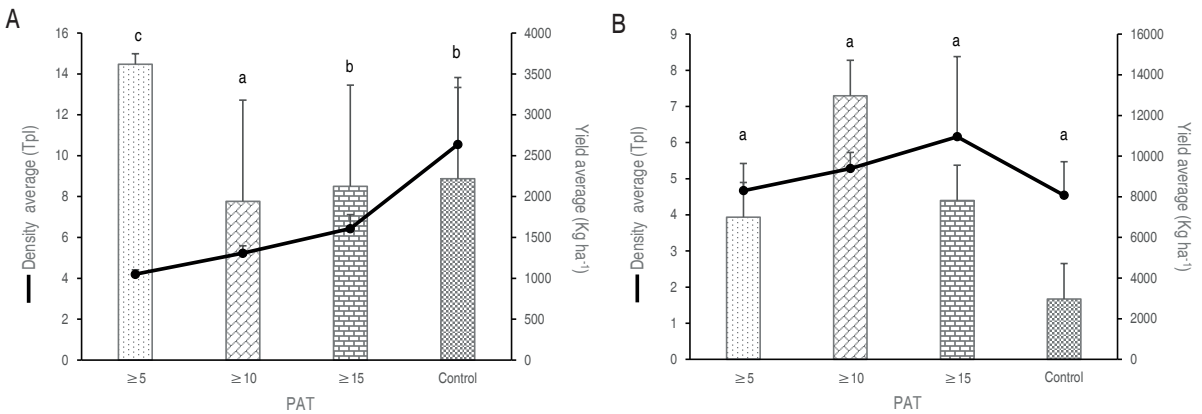

Figure 4. Preliminary action thresholds (PAT) for the *Frankliniella* cf. *gardeniae* thrips in mango var. *tommy*, related with density average (TpI) and to the yield (kg ha^{-1}), in the semester B-2016, Tolima. A. Guamo; B. San Luis.

The damaged unit per insect in the production unit (b) (damage caused by thrips per inflorescence per tree) was estimated by linear regression of the density of thrips by the yield, which allowed establishing the loss function as:

$$Y = 3{,}496.35 - 220.17x \quad (P = 0.0257,\ \text{RMSE} = 935.14,\ R^2 = 0.35)$$

In this sense, 220.17 kg can be the loss if, on average, a thrip per inflorescence per tree is added (Figure.4A). From the calculated EIL (11 TpI), the AT was calculated considering the population growth rate (TPGR) of 0.55 thrips per inflorescence per day obtained from a linear trend between the days after emergence and the thrips per inflorescence per tree (P=0.0203, RMSE=2,106, R^2=0.87). Another variable was the time between sampling (TBS) that was considered as 8 days; the other variable of product efficiency (K) was the same used in the EIL. Finally, the AT for the *tommy* variety in the municipality of Guamo was 7 TpI (Table 1), estimated employing the following equation:

$$AT = (EIL - (TPGR \times TBS)) \times K$$
$$AT = (10.64 - (0.55 \times 8)) \times 0.80$$
$$AT = 7.12 \simeq 7 \text{ TpI}$$

The insect populations are dynamic and variable, generally conditioned by biological characteristics, climatic conditions, geographic environment, and human interference (Lin *et al*., 2015). A factor that favored thrips populations in Guamo and caused AT in var. *tommy* is the temperature since that locality registered a higher average temperature (28.65±0.22 °C) than San Luis (27.24±0.20 °C) during the sample period. This result coincided with the results obtained by Sierra-Baquero *et al*. (2018), who reported a direct relationship between mango thrips populations (var. *tommy*) and temperature (r=0.48), as well as Lin *et al*., (2015), who recorded that the thrips population (*S. dorsalis*) in mango increased with high temperatures.

Table 1. Calculation of EIL and AT for the *Frankliniella* cf. *gardeniae* thrips in mango var. *tommy*, during the semester B-2016, in the municipality of Guamo (Tolima).

Estimated parameter	Total control cost (COP ha^{1})	Price (COP kg^{-1})	Damage index (kg per ha per thrips per inflorescence per tree)	Control efficiency (%)	Result (thrips per inflorescence per tree)
EIL var. *Tommy*	1,874,286	1,000	220.17	0.80	10.64 ≈ 11
Estimated parameter	**EIL (thrips per inflorescence per tree)**	**TPGR (thrips per inflorescence per tree per day)****	**TBS (days)**	**Control efficiency (%)**	**Result (thrips per inflorescence per tree)**
AT var. *tommy*	10.64	0.55	8	0.80	7.12 ≈ 7

EIL: economic injury level; AT: action threshold; TPGR: thrips population growth rate; TBS: time between samplings.

Therefore, the AT of the pests varies according to the specific conditions of the localities and varieties (Jaramillo-Barrios *et al*., 2020). For example, in Taiwan, the AT of the thrips (*Scirtothrips dorsalis*) in the mango was established at >17 thrips per trap every 3 days (Lin *et al*., 2015). In Mexico, the AT for *Frankliniella* spp. in mango (var. *manila*) was calculated at 10 TpI (García-Escamilla *et al*., 2016), the same authors reported that *Frankliniella* genus in mango var. *ataulfo* is a pest that in high populations (600 TpI) decreased the yield, causing economic losses. In addition to the action threshold, it is very important to know the most susceptible phenological stage of the plant to the attack of the insect under consideration to perform an efficient control of the pest. In mango, thrips diminish their populations naturally when the food source (inflorescence) is exhausted (García-Escamilla *et al*., 2016). Thus, to carry out chemical applications after flowering or when mango fruits have a size greater than 8 mm is not necessary (Durán, 2012; Morse and Hoddle, 2005; Sierra-Baquero *et al*., 2018).

The behavior of thrips as an occasional pest in mango suggests that some species of the *Frankliniella* genus are not specific to it because the population of the control treatment solely exceeded the action threshold in the flowering season. Therefore, chemical controls should be used only in the flowering stage to the beginning of the fruit set, and it is recommended to use specific or low toxicity insecticides (García-Escamilla *et al*., 2016). In this study, specific insecticides for thrips control were used (spinetoram and sulfoxaflor), which allowed a major control of the pest populations. According to Rocha *et al*. (2012), the management of thrips in mango (var. *ataufo*) should not be carried out with broad-spectrum insecticides such as dimethoate and deltamethrin because they do not have effectiveness in the pest control and harms their beneficial entomofauna. It is recommended to generate thrips control strategies, which include monitoring, follow up to populations, and cultural and biological practices (Jaramillo-Barrios *et al*., 2020).

CONCLUSIONS

Thrips generated an economic injury level in the *tommy* mango variety in Guamo, from which an action threshold was estimated, the thrips complex (*F. gardeniae*) must be controlled chemically in the period of flowering and beginning of fruit formation. In var. *yulima*, chemical control strategies should not be implemented since thrips did not appear to represent economic damage for this variety. Instead, preventive practices, such as the application of repellents and trap plants, should be implemented, ideally along with the release of biological control agents.

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Factors associated with the technology adoption in dairy agribusiness

Factores asociados con la adopción tecnológica en agronegocios lecheros

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ABSTRACT

The adoption of adequate technologies is essential to improve the performance of different kinds of companies. Although there is literature related to the adoption of technology in dairy agribusiness in developed countries, information about it is scarce in developing countries. The objective of this study was to explore the factors associated with the adoption of technology by dairy agribusiness. A structural analysis was conducted to evaluate the relationships between the internal variables of Antioquia province farms, Colombia, and their technology adoption process. This process was explained by two factors: production-related variables and management-dependent traits. The variables resulting in the greatest impact were pasture improvement, genetics, administration, and technical procedures.

Keywords:
Agricultural development
Productivity
Rural economics
Structural equations

RESUMEN

La adopción de tecnologías adecuadas es esencial para incrementar el desempeño de las compañías. Aunque existe literatura relacionada con la adopción tecnológica en agronegocios lecheros en países industrializados, la información acerca de este fenómeno en países en vía desarrollo es escasa. El objetivo fue explorar los factores asociados a la adopción tecnológica en agronegocios lecheros. Se realizó un análisis estructural para evaluar la relación entre variables internas en granjas del departamento de Antioquia, Colombia y el proceso de adopción de tecnologías. La adopción tecnológica fue explicada mediante dos constructos: producción y gestión, siendo el mejorar los pastos, la genética, la gestión administrativa y los procedimientos técnicos las variables de mayor impacto.

Palabras clave:
Desarrollo agrario
Productividad
Economía agraria
Ecuaciones estructurales

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Technology appropriation helps reducing workload, improves the life quality of farmers, and increases farm performance (Tse *et al*., 2018). Although the use of technology has increased in recent years, its adoption rate by the dairy sector has been slow compared with other industries (Russell and Bewley, 2013).

Investing in dairy farming technologies implies overcoming several challenges. It is necessary to consider the reasons argued for investing or not (Steeneveld and Hogeveen, 2015), the technical efficiency (Steeneveld *et al*., 2012), and its economic consequences (Bijl *et al*., 2007), among others. Lack of analysis of the specific conditions and needs of farmers is a relevant factor that discourages investment (Luvisi, 2016).

Information about the adoption and use of technology by dairy farmers in developing countries is scarce (Janssen and Swinnen, 2017). This includes Colombia, where such lack of information could be related to its low rate of technology adoption (Barrios *et al*., 2019). Therefore, it is necessary to investigate the technology adoption processes by dairy farmers in this country. The results could improve decision making and productive performance in a sector that is still immature regarding organizational issues and business management (Vásquez-Jaramillo *et al*., 2018). The objective of this study was to establish the factors associated with the adoption of technologies by dairy agribusiness. The findings could help increase the effectiveness of research and policy-making agencies for supporting extension programs.

MATERIALS AND METHODS

A survey that included 45 questions distributed in two sections was carried out to determine the factors related to technology adoption. The first section provided the information required for a general understanding of demographics and productive characteristics of dairy agribusiness. The second section focused on those factors that farmers considered important to make decisions on whether or not to adopt a technology. Data were collected between May and December 2018. A total of 280 farmers across eight municipalities of Antioquia province, Colombia, responded to the survey. Stratified sampling by size (Sorge *et al*., 2016) and municipality (Milán *et al*., 2003) was used. The municipalities were: Bello (14), Belmira (25), Donmatías (25), Entrerríos (29), San José de la Montaña (7), San Pedro de los Milagros (61), Santa Rosa de Osos (76), and Yarumal (43).

A Likert response scale with levels from 1 ("Not important") to 5 ("Very important") was used to evaluate the relationship between the process of technology adoption and the farm operational and management variables. The statistical procedure included an exploratory factor analysis using the psych library (Revelle, 2017) of the R-project software (R Core Team, 2018) and a model of structural equations. According to the Schmid-Leiman procedure (Revelle, 2017), only variables with Cronbach's alpha values higher than 0.70 and a factorial load higher than 0.25 were included in the model. Model fit was validated by a Root Mean Square Error of Approximation (RMSEA) less than 0.1 and both Comparative Fit Index (CFI) and Goodness of Fit Index (GFI) greater than 0.9 (Cangur and Ercan, 2015) using the lavaan library (Rosseel, 2012) of the R-project software (R Core Team, 2018).

RESULTS AND DISCUSSION

The average age of farmers was 47±12 years, and they had 25±13 years of experience in dairy farming (Table 1). Both traits are known to favor productivity, considering that experiential knowledge facilitates decision-making (Cuartas-Martínez *et al*., 2018). However, this knowledge was not accompanied by academic training; on average, they attended through eighth grade, which means they did not complete high school. This finding is known to discourage "Management by Competencies" and limits individual and organizational learning (Pardo and Díaz, 2014). This could explain why the Colombian dairy sector has been focused for years on its survival rather than growth and business development (Barrios *et al*., 2016).

Table 1. Relevant characteristics of dairy agribusiness in Antioquia, Colombia.

Item	Mean±SD	Median
Age of farmers (yrs)	47±12	48
Experience of farmers in the dairy business (yrs)	25±13	25
Number of full-time employees	1.99±1.83	1
Distance from the main town (km)	10.61±6.82	10

Regarding organizational characteristics, 70% of producers work in their own farms (Table 2). This factor, added to the fact that in 62.85% of cases there was a successor to the business, could promote the adoption of new technologies by this type of organization since there is certainty about the fate of the property in the long term. It could be related to the fact that 74.64% of farmers have used medium or long-term financing, a figure higher than that found by Rodríguez *et al*. (2015), who reported, for the same region, a 38% credit-access rate. In that study, they also reported that the technical assistance rate was 50%, meaning that coverage of technical assistance and technology transfer programs have improved in recent years, reaching 89.64% for the surveyed organizations. These results are positive because such programs help to guide farmers towards appropriate decision-making processes (Cerón-Muñoz *et al*., 2015).

Table 2. Organizational characteristics of dairy agribusiness in Antioquia, Colombia.

Item	%
There is a successor to the business	62.85
The farmer is the owner of the farm	70.00
The farmer has used medium or long-term financing	74.64
The farmer is associated with a cooperative	53.57
The farmer regularly attends technology-related fairs and other events	90.36
The farmer has received technical assistance from the government	89.64

Improvement of milk quality, Pastures, and Herd genetics were the most important aspects that influence a farmer's intention to adopt technologies, averaging 4.58±0.59, 4.57±0.61, and 4.52±0.63, respectively (Table 3). A tendency to favor the adoption of technologies related to purely technical aspects is frequent in the dairy sector, where it is common to find higher adoption rates of "hard" technologies in comparison to those associated with knowledge management and improvement of procedures and management methods (Barrios *et al*., 2016).

Human management was the least relevant variable when deciding on the adoption of technologies (3.09±1.41). This result disagrees with the report by Steeneveld and Hogeveen (2015), who found that investment in dairy technology significantly reduces labor, decreases production costs, and improves the life quality of farmers.

Table 3. Factors that influence technology adoption by dairy agribusiness in Antioquia, Colombia[1]

To improve	%			Mean±SD
	Not important	Important	Very important	
Milk quality	0.4	8.2	91.4	4.58±0.59
Pasture quality	1.1	7.8	91.1	4.57±0.61
Herd genetics	0.4	8.9	90.7	4.52±0.63
Production cost	2.5	17.5	80.0	4.39±0.85
Administrative procedures	5.7	15.7	78.6	3.93±0.90
Supplies	3.9	24.3	71.8	4.02±0.93
Equipments	6.1	32.8	61.1	3.85±1.06
Technical procedures	6.8	33.9	59.3	3.76±0.98
Recognition of the farm in the market	18.2	26.8	55.0	3.54±1.28
Human management	24.4	36.2	39.4	3.09±1.41

[1]Response categories: 1 = Not important, 3 = Important, and 5 = Very important.

Variables with no statistical significance were eliminated after the exploratory analysis. Thus, only ten variables grouped into two factors were included in the structural equation model. This helped to identify the structure of the relationships between variables and conformed factors (Table 4). According to the common characteristics of variables grouped in each factor, it was possible to name Factor 1 as a production-related factor, while Factor 2 included variables related to business management.

Table 4. Variables associated with technology adoption by dairy agribusiness in Antioquia, Colombia

Factor	Variable	Factor loading	Mean±SD	Cronbach's alpha
Production	Pasture quality	0.70	4.57±0.61	0.70
	Herd genetics	0.65	4.52±0.63	
	Production costs	0.59	4.39±0.85	
	Equipment	0.53	3.85±1.06	
	Milk quality	0.51	4.58±0.59	
Management	Administrative management	0.69	3.93±0.89	0.71
	Technical procedures	0.66	3.76±0.98	
	Human resource management	0.58	3.09±1.41	
	Supplies	0.57	4.02±0.93	
	Farm recognition in the market	0.43	3.54±1.28	

The structural equation model resulted in a Cronbach's alpha value higher than 0.7 for the proposed factors (Table 4), with 0.072 RMSEA, and fit indexes of 0.921 and 0.948 for the CFI and the GFI, respectively. This indicates the internal consistency of the scale and a good fit of the model (Cupani, 2012).

Factor "Production" included the following variables: *Pasture*, *Herd genetics*, *Production costs*, *Equipment*, and *Milk quality* (Figure 1). *Pastures* and *Herd genetics* were the most representative variables, with 0.7- and 0.65-factor loads, respectively. This could be due to the fact that forage quality and genetic improvement are related to dairy herd planning (Múnera-Bedoya *et al*., 2018; Cerón-Muñoz *et al*., 2017) which is considered a strategic tool linked to technology adoption. It is important to mention that variable *Production costs* (with 0.59 factorial load) was one of the aspects that determined the adoption of technology, which could promote the analysis of costs in this sector, considering that this industry has presented historically low rates of economy diagnostic at the organizational and sectorial level (Barrios and Olivera, 2013).

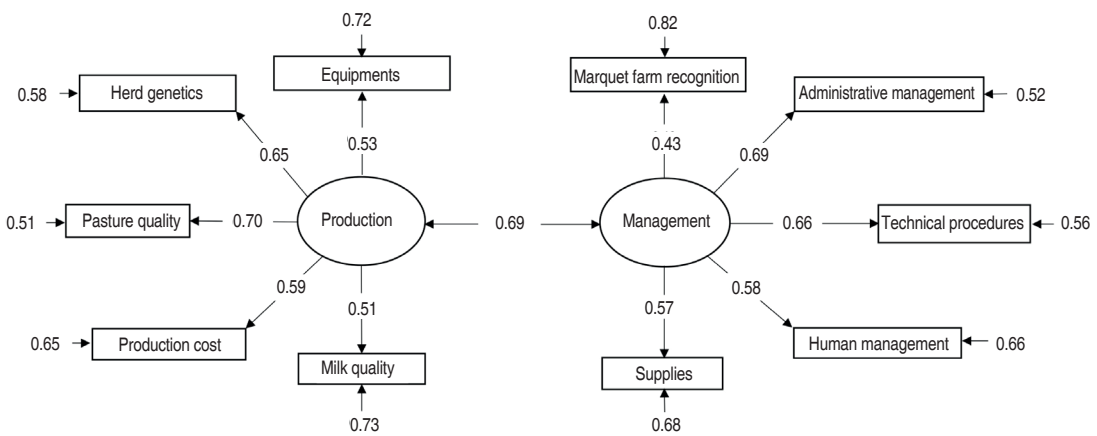

Figure 1. Structural equation model for factors associated with technology adoption by dairy agribusiness in Antioquia, Colombia.

The second factor, "Management," was impacted by variables: *Administrative management*, *Technical procedures*, *Human resource management*, *Supplies*, and *Farm recognition in the market.* The variables with the greatest weight in the factor were *Administrative management* (0.69 factorial load) and *Technical procedures* (0.66 factorial load). These variables are related to document management, which denotes the importance of information traceability for adequate analysis and subsequent decision making (Londoño *et al.*, 2016).

Although variable *Farm recognition in the market* had the lowest factor load (0.43), it is important to highlight how the decision to adopt technology is positively influenced by the image that the farm could project in the sector. This result is somewhat unusual, considering that this market has a regional oligopsonic structure where, in normal conditions, the total production of milk is sold (Von Keyserlingk *et al.*, 2013). This could be associated with having a productive system with technology levels in line with or higher than the industry standard, which in the long term can be considered as a sustainability strategy for the organization (Adefulu, 2015).

CONCLUSIONS

The intention to adopt technologies by dairy farmers is influenced by factors inherent to production and business management traits. Improving technical aspects such as pastures, herd genetics, production costs, equipment, and milk quality can affect the overall production factor. Additionally, the Management factor is influenced by the intention to improve administrative and technical processes, human resource management, the supply process, and the recognition of the farm in the market.

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Financial analysis of potential *Pinus patula* plantations in Antioquia, Colombia

UNIVERSIDAD NACIONAL DE COLOMBIA

Análisis financiero de potenciales plantaciones de *Pinus patula* en Antioquia, Colombia

doi: 10.15446/rfnam.v73n2.82833

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ABSTRACT

The establishment of commercial forest plantations requires the selection of sites where reasonable profitability can be attained. A financial analysis was made for the identification of the most suitable areas for the establishment of new *Pinus patula* plantations in the central region of Antioquia, Colombia. The analysis was performed assuming basic silvicultural treatments at the establishment but no management during the entire rotation period. Volume yield data at the stand level was obtained from a previously fitted model that uses biophysical variables and stand density as predictors. The estimated stand volume, a detailed cash flow, and a derived stumpage price were combined to perform a financial analysis. The Land Expectation Value (LEV) and Internal Rate of Return (IRR) at the optimal rotation age, along with their spatial variation, were calculated in this study. Results suggest that the estimated volume and the current stumpage price are not sufficient to guarantee reasonable profitability for new timberland investments. While the LEV was negative, the IRR was in the range 4.1±1.5%, which is less than the discount rate of 6.8% used in the financial analysis. However, a positive LEV and an IRR at 8% would be achieved if forest productivity increases by 20% because of silvicultural practices or costs reduction in a similar proportion (obtaining IRRs up to 8.4%). Moreover, if the government provide subsidies, the IRR would increase up to 10.3% (without requiring an increase in productivity or a decrease in costs) on sites with high growth potential (mean annual increment greater than 16 m^3 ha^{-1} $year^{-1}$), and close to the mills (less than 45 km radii).

Keywords:
Land expectation value
Rate of return
Stumpage price
Timberland investments

RESUMEN

El establecimiento de plantaciones forestales comerciales requiere seleccionar sitios que garanticen una rentabilidad razonable para inversiones forestales. Se realizó un análisis financiero con el fin de identificar las áreas con mejor aptitud para el establecimiento de nuevas plantaciones de *Pinus patula* en la zona central de Antioquia, Colombia. El análisis se realizó asumiendo tratamientos silviculturales básicos en el establecimiento, pero ningún manejo durante el período de rotación. Información de rendimiento forestal en volumen a nivel de rodal se obtuvo de un modelo previamente ajustado, el cual depende de variables biofísicas y de la densidad de rodal. El volumen estimado a nivel de rodal, un flujo de caja detallado, y el precio de la madera en pie, se usaron en el análisis financiero. Se calcularon como criterios de bondad de inversión el Valor Económico del Suelo (VES) y la Tasa Interna de Retorno (TIR) a la edad óptima de rotación, así como su variación espacial. Los resultados sugieren que el volumen estimado de madera y los actuales precios no son lo suficientemente altos para garantizar una rentabilidad razonable para el establecimiento de nuevas plantaciones. Mientras el VES estimado fue negativo, la TIR encontrada se ubicó en el rango 4,1±1,5%, la cual es menor a la tasa de descuento de 6,8% usada en el análisis financiero. No obstante, valores positivos de VES pueden alcanzarse si se realizaran tratamientos silviculturales que conlleven a un aumento de la productividad forestal de 20%, o a una reducción de costos de la misma magnitud, alcanzando una TIR de hasta 8,4%. En un escenario de subsidios a la reforestación proporcionados por el gobierno, la TIR podría incrementar hasta 10,3%, sin requerir aumentos en la productividad o disminución de los costos, en sitios con alto potencial de crecimiento (incremento medio anual mayor a 16 m^3 ha^{-1} $año^{-1}$), y localizados a un radio de 45 km de los centros de transformación.

Palabras clave:
Valor económico del suelo
Tasa de retorno
Precio en pie
Inversiones forestales

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Forest plantations are seen as an attractive investment option compared to alternative investments such as agriculture and livestock. Moreover, increasing global demand for wood (FAO, 2018) encourages timber production from forest plantations. Tropical countries can meet this demand (e.g., Colombia) where high rates of forest growth are possible as a result of more favorable environmental conditions, such as high and constant radiation, and well-distributed rainfall (Cubbage *et al*., 2007). More than seven million hectares (ha) have been identified with high potential for the establishment of new industrial forest plantations in Colombia (UPRA, 2015). However, 360,000 ha have only been established (PROFOR, 2017).

Site selection analysis for establishing new commercial plantations is crucial to guarantee efficient use of land resources. In Colombia, site selection for establishing forest plantations has been carried out mainly through descriptive biophysical analyses, including variables such as temperature, precipitation, and soil depth (UPRA, 2015). Financial analyses of forest plantations have also been developed using the Net Present Value (NPV), Land Expected Value (LEV) and Internal rate of Return (IRR) for plantation species in the Andean Region (*Alnus jorullensis*, *Cordia alliodora*, *Pinus patula*, *Cupressus lusitanica*, *and Eucalyptus grandis*) (Gutiérrez *et al*., 2006), and the Caribbean region (*Tectona grandis*) of Colombia (Restrepo and Orrego, 2015). Notwithstanding, efforts to identify potential areas for new forest plantations have not had a commercial focus that allows for the identification of areas with higher profitability (UPRA, 2018). So far, an analysis combining both financial analysis and biophysical site selection has not yet been undertaken for any species in the country.

A spatially explicit analysis was developed, aiming to narrow the gap between the traditional biophysical site selection and the typical financial analyses commonly developed in Colombia. This analysis included the determination of the potential timber production in these areas and the estimation of the stumpage price. The study was focused on *Pinus patula* in Antioquia (Colombia), a region identified as having high potential for developing productive rural activities under a post-conflict scenario. Although areas with high potential for the establishment of forest plantations have been previously identified in Antioquia, the main objective of this research was to assess how factors such as distance to mills, hauling costs, and stumpage prices will influence the potential profitability of timberland investments. This research gives insights into how these variables might affect both LEV and IRR. Productivity and distance thresholds, which make LEVs and IRRs attractive for *P. patula* in Antioquia, were also identified. It is assumed that the implicit inclusion of socioeconomic variables for site identification may not be enough to select the most profitable areas. The variables previously described will likely change the selected profitable areas since financial factors can have a stronger influence than biophysical factors from an investment perspective.

Results will guide future timberland investments in the region and guarantee efficient use of resources. The methodology applied relies on geographical information systems, international databases (e.g., Worldclim, SoilGrids, SRTM data), and local information obtained from the main forest products companies currently operating in the region. With additional information provided by the forestry sector at the national level, a broader analysis could be further carried out for the entire country considering other relevant forest species, becoming a valuable tool that could contribute to the current national government plans of commercial forest plantations expansion in Colombia. This analysis can be considered as a baseline analysis based on the current timber market conditions in Colombia. An emerging market where informality tends to be a prevailing feature, and there are not institutions responsible for collecting and analyzing relevant information on timber prices, product specifications, establishment and management costs, and trading volumes.

MATERIALS AND METHODS

Study area

A total of 2.2 million ha have been classified as suitable areas for the establishment of commercial forest plantations in Antioquia (UPRA, 2015). Tracts with at least 2,000 ha within this region were selected as the study area in this research. The size of the selected tracts was consistent with the minimum area required to guarantee profitability for a forest project in Colombia (PROFOR, 2017). Moreover, the optimal altitudinal range for *P. patula*, 2,000-2,800 masl (Perry, 1991) was considered, leading to a study area of 115,655 ha distributed across

27 tracts with an average size of 4,283 ha. The current land use of these tracts is mainly agriculture and livestock, with predominant private ownership. The financial analysis was performed for each of the tracts located in the study area. Six different mills located in the study area are responsible for most of the demand for timber (Figure 1).

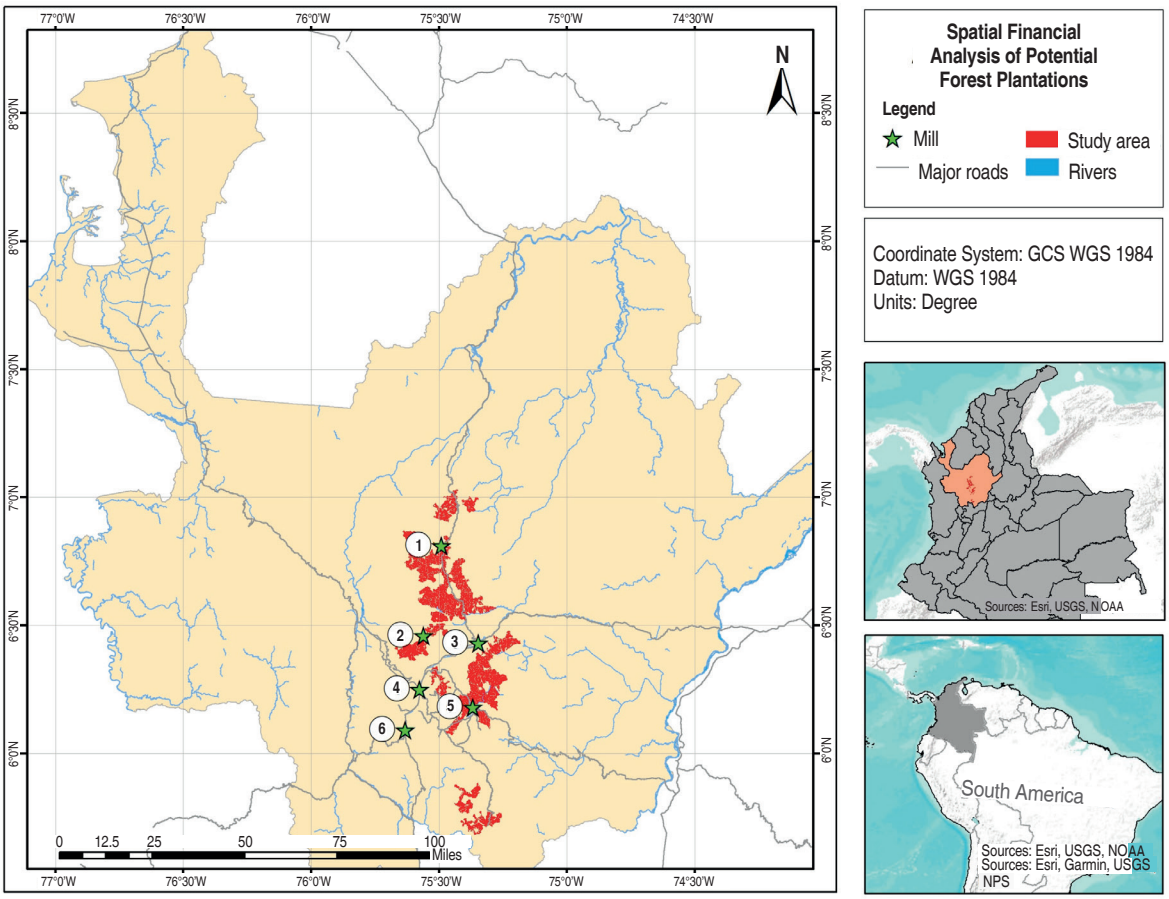

Figure 1. Study area. 1: Cuivá Plains, 2: Northern Plateau, 3: Barbosa, 4: Medellín, 5: Eastern Antioquia, 6: Caldas. Source: own elaboration.

Volume estimation

A yield model fitted by Restrepo *et al.* (2019) was used to estimate forest yield for each tract of land in the study area. In this model, the parameters are expressed as a function of stand density and biophysical variables such as slope, soil pH, mean annual temperature and mean annual precipitation. This stand volume equation is a Bertalanffy-Richards type model that was estimated using 1,119 temporary plots of unthinned, unmanaged, and genetically unimproved *P. patula* plantations in Antioquia, Colombia. Basic silvicultural prescriptions such as fertilization and vegetation control at the establishment were considered. The model can be written as:

$$y = \phi\left[1-exp(-\beta(Age))\right]^{1/1-\gamma} \quad (1)$$

with:

$$\hat{\phi}=235.994-112.423(pH)+2.193(S)+0.176(N) \quad (2)$$

$$\hat{\beta}=-0.123+0.545(T_e P_r) \quad (3)$$

$$T_e P_r=\frac{T_e}{P_r}x100 \quad (4)$$

$$\hat{\gamma}=0.92 \quad (5)$$

Where y is the yield (m^3 ha^{-1}), *Age* is the stand age (years), and ϕ, β, and γ are parameters that denote the asymptote, the intrinsic growth rate, and the shape of the yield curve, respectively. The estimated value for γ does not depend on environmental variables. The parameter ϕ is estimated as a linear function of an intercept, and soil *pH* (a dummy variable that equals one if *pH* is in the range 5.1-6, and zero if *pH* is in the range 4.1-5), slope (*S*, degrees), and stand density (*N*, trees per hectare) (Equation 2). The parameter β was estimated as a linear function of an intercept, and the mean annual temperature (T_e, °C) by the mean annual precipitation (P_r, mm) ratio (T_e P_r) (Brown and Lugo, 1982; Restrepo *et al.*, 2019).

International climate and soil databases available on raster format were the main sources of information for obtaining spatially estimates of ϕ and β. Soil *pH* was obtained from the SoilGrids database, a global compilation of soil profile data layers at 1 km resolution (Hengl *et al.*, 2014). The slope of the terrain was calculated using elevation data from the digital elevation model (DEM) developed by the Shuttle Radar Topography Mission (SRTM), and available at a spatial resolution of 30×30 m (Farr *et al.*, 2008). The mean annual temperature and the annual precipitation were obtained from the Worldclim database, version 2.0, and available at a 1 km resolution (Fick and Hijmans, 2017). Forest yield was estimated using the average value for each biophysical variable and each tract. Merchantable volume was assumed to be 95% of the stand volume, assuming three different products can be obtained from the harvest: roundwood large size, roundwood medium size, and pulpwood.

Stumpage price

The stumpage price can be estimated as the timber price at the mill minus hauling and harvesting costs, using the residual price methodology (Giudice *et al.*, 2012). The equation proposed by Stone (1998), in which the stumpage value is a decreasing linear function of the distance, was adapted in this study to estimate the stumpage price for each tract. All the values were calculated in US dollars (USD) using an exchange rate of 2,854 Colombian pesos (COP) per USD.

Three main timber classes (*k*) were considered: roundwood large size, roundwood medium size, and pulpwood. A blended stumpage price was calculated, corresponding to the average timber price of each product weighted by the proportion of that product in a typical harvest (Table 1).

Table 1. Product class specifications and pricing.

	Specifications	Average proportion per harvest (%)	Average delivered price (USD t^{-1})
Roundwood large size	Diameter >16 cm in the smallest section	58	78.32
Roundwood medium size	Diameter between 11-15 cm in the smallest section, length >6 m	19	72.68
Pulpwood	Diameter >5 cm	23	42.92

The blended stumpage price for each tract in the study area was calculated as follows:

$$\pi_{B_i} = p_B - c - hd_i - f_r \quad (6)$$

Where π_{B_i} is the blended stumpage price (USD t^{-1}) for the tract *i*, p_B is the blended delivered price (69 USD t^{-1}), *c* is the harvesting cost at 21 USD t^{-1}, *h* is the hauling cost at 0.18 USD t^{-1} km^{-1}, d_i is the distance from the tract's nearest existing road to the nearest mill (km), and f_r is the forest road construction cost (diluted to two rotations), obtained as an average cost per ton of wood at 2.2 USD t^{-1}. Harvesting and hauling costs correspond to average harvesting costs (including loading) for the region and include skyline cables and animal-powered logging. The information required for estimating the stumpage price was largely obtained from interviews with professional employees of the main forest products companies located in the study region.

Stumpage price was estimated using the ArcGIS software 10.3. Cost distance analysis was used to determine the transportation cost on existing roads (d_j). This analysis allowed for the identification of the least-cost path for timber transportation, optimizing the use of the existing roads, and minimizing the number of new roads to be constructed.

Financial Analysis

Two financial criteria, the LEV and the IRR, were used to evaluate the potential investments in the area of study. LEV can be defined as the NPV of the cash flow of a timberland investment by assuming infinite rotations, with no changes in economic conditions (Samuelson, 1976; Chang, 1984). The discrete version of LEV was used in this study (Clutter *et al*., 1983).

$$LEV = \frac{\sum_{j=0}^{t} CF_j (1+i)^{t-j}}{(1+i)^t - 1} \qquad (7)$$

Where *LEV* is the Land Expectation Value (USD ha^{-1}), CF_j is the cash flow after tax (incomes minus costs) at year *j* (USD ha^{-1}) with a maximum financial horizon time of 20 years, *i* is the discount rate at 6.8% (Mendell and Sydor, 2006), and *t* is the rotation age (years). The financial horizon was defined to cover all the expected optimal rotations based on previous research on *P. patula*, suggesting a financial optimal rotation age between 12 and 14 years (Restrepo *et al*., 2012).

For each tract, an iteration process was implemented to evaluate different rotations (*t* from 1 to 20) to choose the *t* that maximizes the LEV, thereby identifying the optimal rotation age (*T*). The R software version 3.4.4 was used for this analysis (R Core Team, 2018). Likewise, the IRR at *T* for each tract was estimated with the FinCal package in R (Yanhui, 2016).

Costs in Equation 7 were consolidated as the average establishment and management costs for a typical *P. patula* stand in Antioquia. The cash flow used was consolidated after analyzing and compiling the average costs provided by the main forest products companies. All pre-planting and establishment activities, as well as the technical assistance and insurance, were included. Thinning and pruning were not included. A summary of the costs for the first five years is presented in Table 2. The detailed costs are presented in Appendix 1.

Table 2. Costs of establishment and management for *P.patula* in Antioquia. Source: own elaboration.

Year	Cost (USD ha^{-1} year^{-1})	Activities
0	1,304	Land preparation, vegetation clearing, fertilizer application, planting, phytosanitary control, forest road maintenance, replanting, fire protection.
1	447	Competing vegetation control, fertilization, phytosanitary control, forest road maintenance.
2	415	Competing vegetation control, phytosanitary control, forest road maintenance.
3	194	Competing vegetation control, phytosanitary control, forest road maintenance.
4	62	Phytosanitary control, forest road maintenance.
5	58	Phytosanitary control, forest road maintenance.

Costs and incomes were varied to conduct a sensibility analysis. Factors from 0.7 to 1.3 were applied to both costs and incomes, and the LEV and IRR variations, *ceteris paribus*, were assessed. The 95% prediction interval of the volume estimates is within +/-30% of the mean yield curve (Restrepo *et al*., 2019). Besides, although the volatility in the timber market in Colombia has not been assessed, other authors have found volatility of ~23% for

more mature markets (Restrepo *et al*., 2020). Therefore, variations between +/-30% seemed reasonable for this analysis. Another scenario evaluated was the tax exemption for newly registered forest plantations approved by the Colombian government (Congreso de la República de Colombia, 2016). This tax corresponds to 33%, and that level of exemption may have a substantial effect on the profitability of the investment. All new plantations registered with the environmental authority can apply for this exemption.

Scenarios considering simultaneous changes in costs and incomes were evaluated. A total of 169 scenarios were used to evaluate how sensitive the LEV and IRR were to simultaneous changes in costs and incomes. Factors in the range 0.70-1.30 and increments of 0.05 were applied to costs and incomes, with all possible combinations defining the scenarios.

RESULTS AND DISCUSSION

Estimated potential yields at rotation age were in the range of 183-257 $m^3 ha^{-1}$ (Figure 2), values that correspond to mean annual increments (MAIs) in the range of 9-18 $m^3 ha^{-1} year^{-1}$. The estimated MAI was low compared to other studies (26-30 $m^3 ha^{-1} year^{-1}$) (López *et al*., 2010; Restrepo *et al*., 2012). The model used reflects the potential yield for unmanaged stands, which can explain the lower estimated MAIs. Nevertheless, the model provides a conservative estimate of volume yield compared to that obtained from plantations in Colombia with some genetic improvement and silvicultural treatments such as thinning. This model leads to a conservative financial analysis that can be considered as a reasonable lower bound for any financial decision.

Derived stumpage price varied according to the distance to the mills (Figure 3). Stumpage prices in the tracts located around the Northern Plateau mill were in the range 42-45 USD t^{-1}, whereas for the tracts located around the Cuivá Plains were in the range 35-45 USD t^{-1}. An average stumpage price of 40.4±4.9 USD t^{-1} was estimated for all the tracts in the study area, varying from 29 to 45 USD t^{-1}. This result

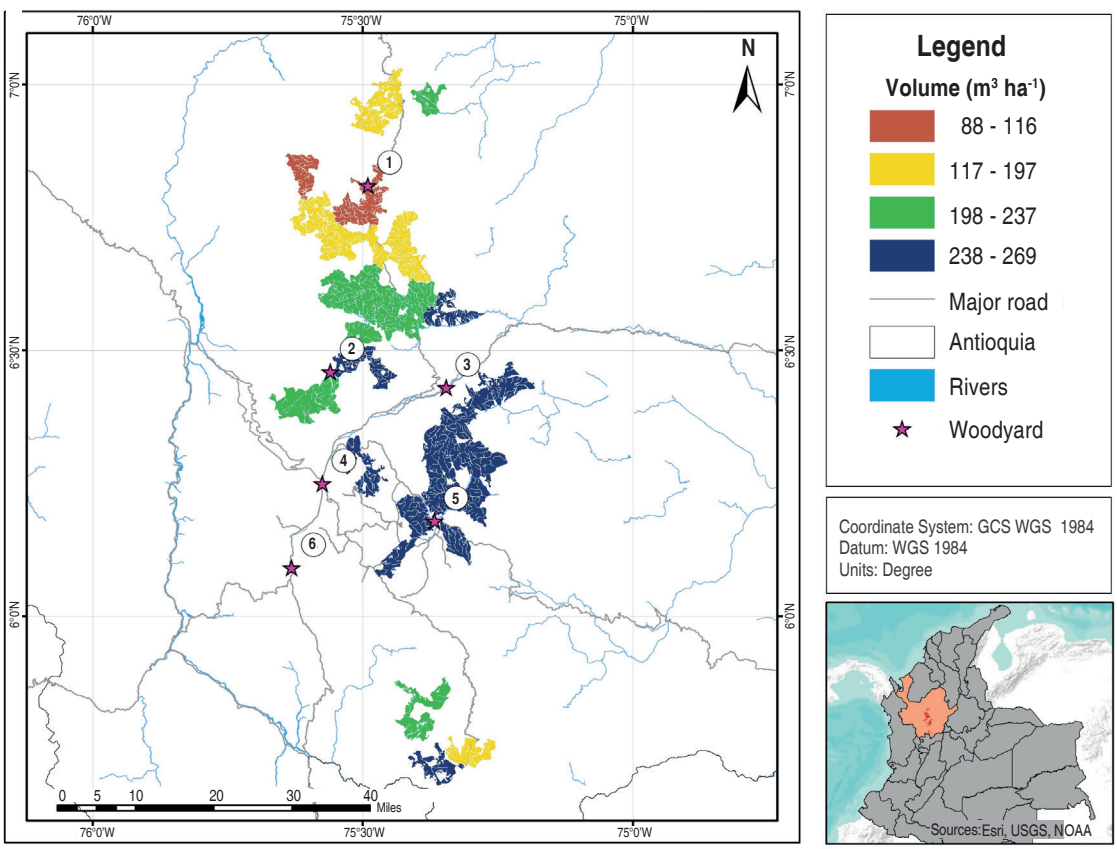

Figure 2. Estimated yield at age 15 years.

was consistent with a 23-42 USD t^{-1} range provided by the main forestry companies operating in the region.

In the Andean region, stumpage prices between 20-53 USD t^{-1} and 53-60 USD t^{-1} have been reported for pulp and sawtimber, respectively, with variations depending on location concerning the woodyard, species, dimensions, and specific conditions of local supply and demand (CIIEN, 2011). The stumpage price found in this study can be considered high compared with stumpage prices in other more mature markets like in the southeast United States, where stumpage prices of 11, 17, and 24 USD t^{-1} are reported for pine pulpwood, chip-n-saw, and sawtimber, respectively (TimberMart-South, 2019). Nevertheless, a lower profit margin can be obtained in Colombia due to the substantially higher transportation costs.

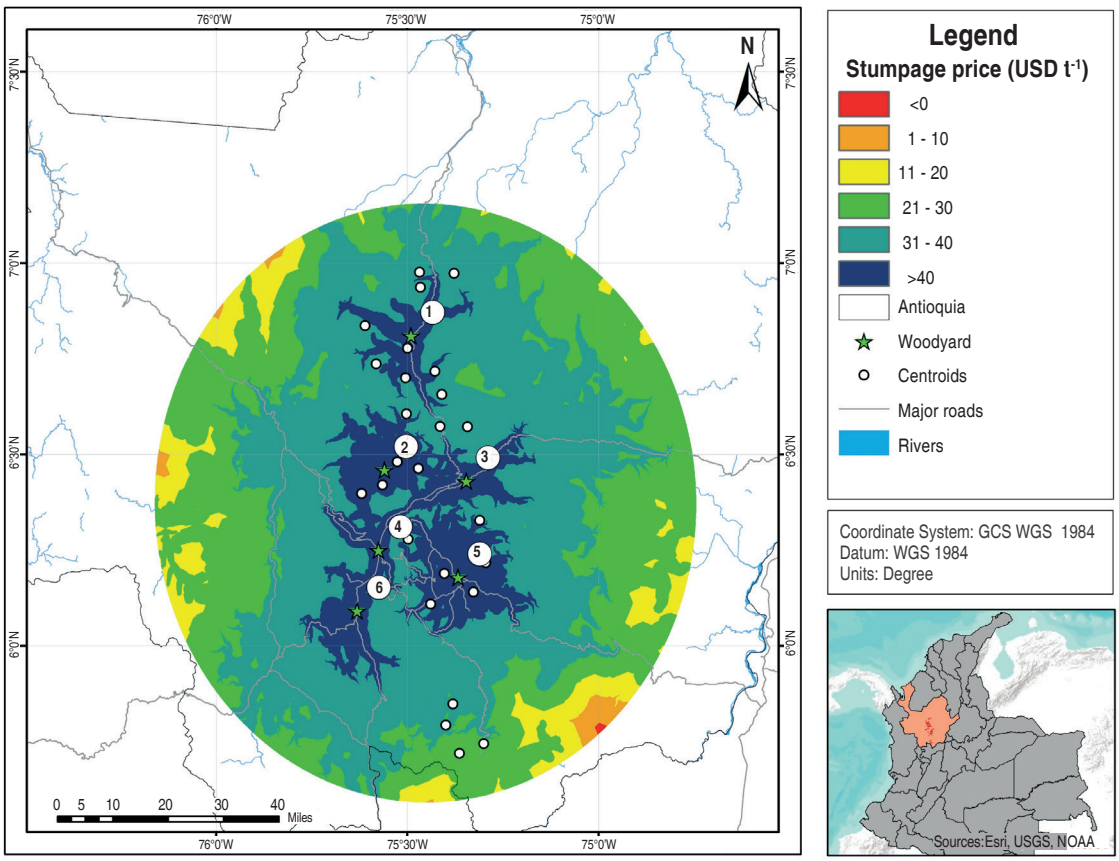

Figure 3. Estimated stumpage price.

Compared to regional markets, it is estimated that for Pinus, the production costs are 40% higher in Colombia than in Brazil (UPRA, 2018a). A high proportion of these costs is attributed to transportation costs, which in Colombia can vary between 12-16 USD t^{-1} $(100\ km)^{-1}$ compared to 9 USD t^{-1} $(100\ km)^{-1}$, on average, for other countries (UPRA, 2018a). In this study, the average transportation cost was 18 USD t^{-1} $(100\ km)^{-1}$. Abrupt topography and a mountainous landscape in the Andean region of Colombia, make the establishment of forest plantations challenging and expensive (Cubbage *et al.*, 2010).

Financial analysis

Negative LEVs were found for all the tracts in the study area. This result implies that based on the current costs, timber volumes and timber prices, it is not profitable to establish new forest plantations in the region. An average IRR at 4.1±1.5% was estimated, varying from 1.0 to 6.3%. Although these areas were previously

classified as zones with medium/high potential for forest plantations establishment (UPRA, 2015), the stumpage price and distance to the mills had a substantial influence on the LEV, making these areas not suitable from a financial perspective. Similar conclusions were found by CIIEN (2011) for a biomass production feasibility study in Colombia. Although similar competitive stumpage prices were found, it was also concluded that high transportation costs reduced profitability and discouraged tree harvesting.

In the baseline scenario, the IRRs were low compared to the IRR reported by Cubbage *et al*. (2007) and López *et al*. (2010). The observed differences are likely due to the higher MAI and timber prices used in their studies, lower establishment costs, and additional government subsidy. This subsidy was not included in this research since the government has not provided it during the last two years. A summary of the IRRs reported in similar studies and the estimated values in this study are presented in Table 3.

Table 3. Summary of the Internal Rate of Returns (IRRs) reported in this and previous studies.

IRR (%)	Description	Reference
1.0-6.3 (mean 4.1)	MAI: 9-18 $m^3 ha^{-1} year^{-1}$ All tax included	Present study
4.0-10.3 (mean 7.5)	MAI: 9-18 $m^3 ha^{-1} year^{-1}$ Tax exemption included	Present study
7.5-10	*P. patula* plantations in Antioquia, MAI: 20 $m^3 ha^{-1} year^{-1}$, government subsidy included	(López *et al*., 2010)
10.5-16.9 (mean 14.3)	Pine plantations in South America, MAI: 22-33 $m^3 ha^{-1} year^{-1}$	(Cubbage *et al*., 2007)
3.9-5.7	*P. patula* plantations in Antioquia, MAI: 7-27 $m^3 ha^{-1} year^{-1}$ All tax included	(Restrepo *et al*., 2012)
8.3-10.6	*P. patula* plantations in Antioquia, MAI: 7-27 $m^3 ha^{-1} year^{-1}$ Tax exemption included	(Restrepo *et al*., 2012)
11.2	*P. patula* plantations in Colombia MAI: 19 $m^3 ha^{-1} year^{-1}$ Before taxes rate	(Cubbage *et al*., 2010)

Sensitivity analysis

The scenarios evaluated showed that positive LEVs in the range of 52–235 USD ha^{-1} would be obtained by either a 10% decrease in costs or a 10% increase in incomes. The results showed that LEV tends to be more sensitive to changes in costs than in incomes. A 10% decrease in the costs would increase the LEV by 53%, whereas a 10% increase in the incomes would increase the LEV by 43%. Moreover, the high variability of the LEV among tracts was also found: LEV increased up to 200% (rising from -217 USD ha^{-1} to 235 USD ha^{-1}) or just 17% (rising from -2,170 USD ha^{-1} to -1,794 USD ha^{-1}). The higher variability was identified in those tracts with a combination of high stumpage price and/or high timber production, whereas smaller changes were identified in tracts with low forest productivity and low stumpage price.

When a 10% decrease in costs or a 10% increase in incomes was simulated, the average IRRs for the tracts with positive revenues were 7.2% and 7.1%, respectively. A 20% cost decrease would increase the IRR to 7.5%, whereas a 20% rise income would increase the IRR to 7.4% for those tracts with positive LEV. The average IRR with a 30% decrease in costs was 8.2%, and with a 30% increase in incomes was 7.9%. Table 4 summarizes the LEVs and IRRs found in all of the evaluated scenarios.

Table 4. Summary of sensitivity analysis[a].

Scenario (ID)			All the polygons		Tracts with positive LEV	
			LEV (USD ha^{-1})	IRR (%)	LEV (USD ha^{-1})	IRR (%)
Baseline scenario			-1,203±611	4.1±1.5	---	---
Cost decrease	(1)	10%	-784±630	4.9±1.6	176±77	7.2±0.2
	(2)	20%	-364±650	5.8±1.7	323±237	7.5±0.6
	(3)	30%	60±670	6.8±1.8	570±352	8.2±0.9
Income increase	(4)	10%	-904±691	4.8±1.6	148±85	7.1±0.2
	(5)	20%	-605±772	5.5±1.7	300±238	7.4±0.5
	(6)	30%	-304±853	6.1±1.7	598±310	7.9±0.6
Tax exemption	(7)		454±1,009	7.5±1.8	1,037±637	8.6±1.1

[a]The interval presented corresponds to one standard deviation.

Under the income tax exemption scenario, the LEV was positive for most of the tracts. For these tracts, the average LEV was 1,037 USD ha^{-1} (Figure 4). In this scenario, IRRs increased up to 10.3%. An average increase in 3.4% of the IRR was obtained in this study beacuse of the income tax exemption. Other studies carried out in South America indicated that government subsidies boost timber investments, increasing the rate of return by 2-3% (Cubbage *et al.*, 2007; Bussoni and Cabris, 2010). Well-designed carbon sequestration payment schemes may generate a similar effect.

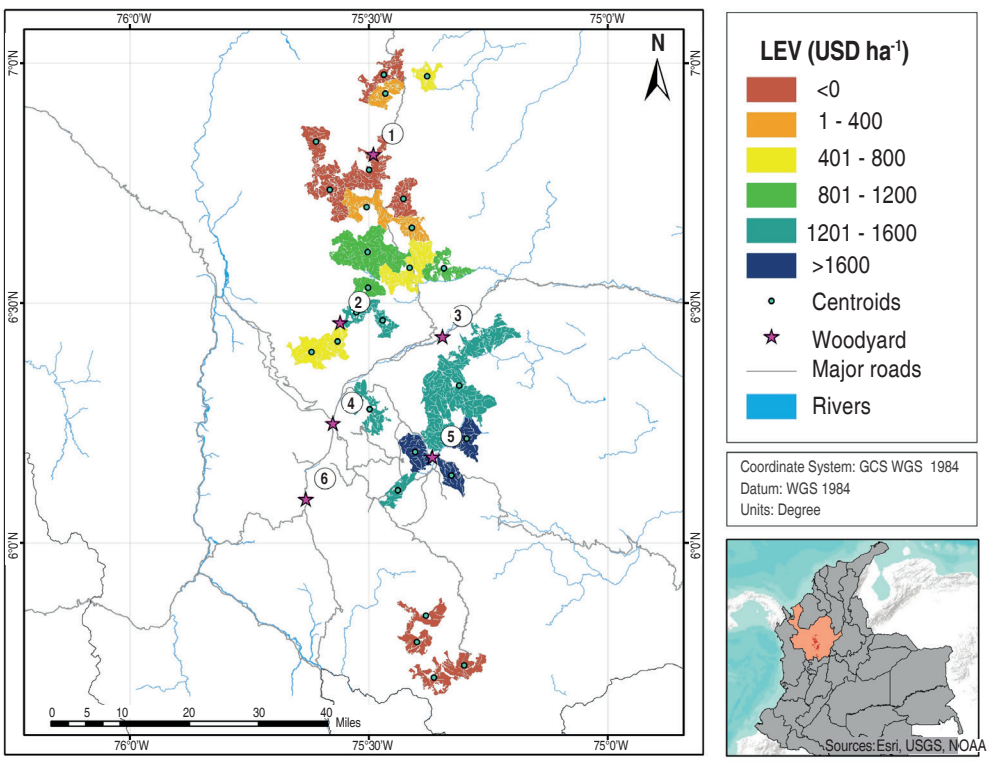

Figure 4. Spatial distribution of the LEV with tax exemption.

Cubbage *et al.* (2007) reported higher average IRRs for pine plantations in South America (10.5-16.9%), a value higher than that reported by López *et al.* (2010) and the value found in this study. Nonetheless, these authors recognize the use of MAIs higher than those reported in the literature, which may lead to higher IRRs. In the present study, similar IRRs (~13%) were obtained after the inclusion of both the tax income exemption and a 25% cost reduction. Restrepo *et al.* (2012) evaluated the profitability of *P. patula* plantations in Antioquia and reported an IRR consistent with the results of this study in the range of 3.9-5.7%. Table 5 shows the average LEV obtained per woodyard with the income tax exemption compared to the baseline scenario. The higher financial returns were obtained for the tracts spatially linked to the woodyard in Eastern Antioquia, a region exhibiting the highest forest productivity. Tracts in the Northern Plateau had the second-highest financial returns, as a result of higher stumpage prices.

Table 5. Average Land Expectation Value (LEV) and Internal Rate of Return (IRR) for each mill.

Mill[a]	Yield[b] ($m^3 ha^{-1}$)	Stumpage price (USD t^{-1})	Baseline scenario		Tax exemption[c]		Rotation Age
			LEV (USD ha^{-1})	IRR (%)	LEV (USD ha^{-1})	IRR (%)	
Cuivá Plains	220	42	-1,551±395	3.3±1.0	385±421	7.5±0.7	15
Northern Plateau	231	43	-885±237	4.9±0.5	979±385	8.5±0.6	16
Barbosa	234	40	-1,089	4.4	656	7.9	17
Eastern Antioquia	236	38	-976±819	4.5±2.1	1,703±311	9.7±0.5	14
Caldas	243	32	-1,790	2.6	---	---	15

[a] It was found through the cost distance analysis that mill 4 (Medellín) would not be a profitable option for transporting the timber products. Therefore, it was excluded in the summary of the results.
[b] At the rotation age.
[c] Only those tracts with positive LEV were included for the average.

Figure 5 shows how IRR is affected by timber production and the distance to the mills. If income tax exemption is considered, the IRR exceeded the discount rate in 18 (67%) of the tracts (positive LEVs were obtained). All these tracts were located within 45 km from a mill. Values between 27 and 71 km have been reported as maximum distance to the mill for ensuring profitability in Colombia (CIIEN, 2011). Nevertheless, not all the tracts situated within 45 km from a mill surpassed the discount rate used in the financial analysis. Forest productivity also had an important influence on the returns. For this species and region, a volume higher than 237 $m^3 ha^{-1}$ (average MAI of 16 $m^3 ha^{-1} year^{-1}$) would guarantee an IRR higher than 6.8% if income tax exemption were considered. However, this is not a static result. Higher distances to the mills can be compensated by higher productivity, as well as lower productivity can be compensated by a shorter distance to the mills. Figure 6 shows similar relationships for the LEV.

Small variations in the optimal rotation age were observed as a result of changes in costs and incomes. The optimal rotation varied from 13 to 15 years for scenarios 1 to 6 in Table 4. By considering the income tax exemption (scenario 7 in Table 4), a higher variation was observed, the optimal rotation age was in the range 13-17 years with an average of 15 years.

Sensitivity analysis – Simultaneous changes

The analysis of scenarios due to multiple changes in costs and incomes suggests that either a 5% reduction in costs or a 10% increase in incomes is required to obtain positive LEVs when simultaneous variations are considered as alternative scenarios. Nevertheless, a positive LEV was obtained for only 1 out of the 27 tracts of the study area when considering the 5% reduction in costs, and when a reduction of the 10% in costs was simulated, three tracts with a positive LEVs were obtained. A reduction of 15% in costs would be required to achieve higher LEVs up to 462 USD ha^{-1}, whereas a 15% increase in incomes would generate LEVs up to 429 USD ha^{-1}. This is a reasonable price to be paid for land in the region, where forest lands are considered marginal after long-term use in agriculture or livestock.

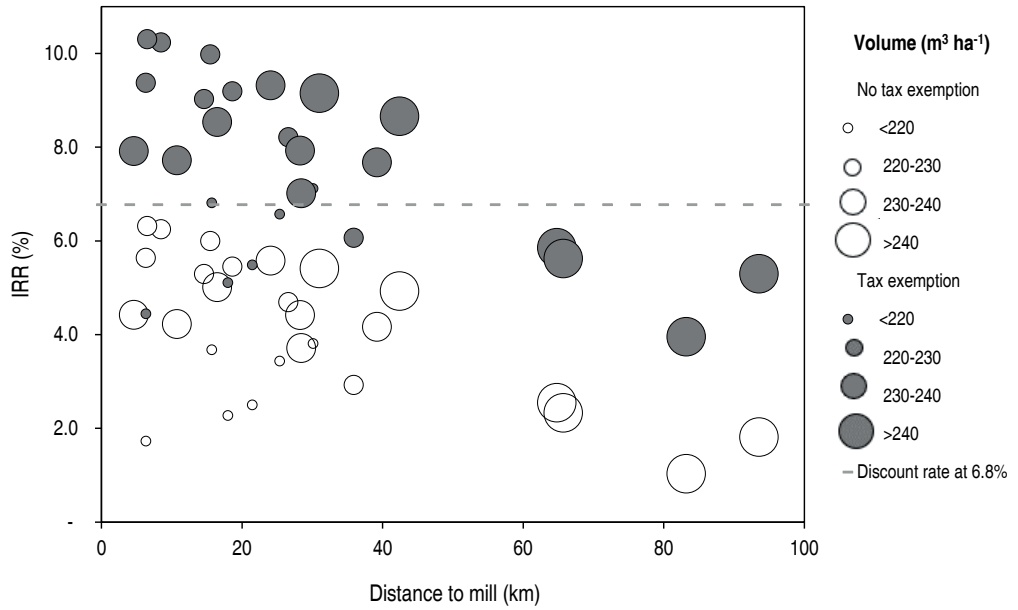

Figure 5. Relationship between the distance to the mill, volume and Internal Rate of Return (IRR).

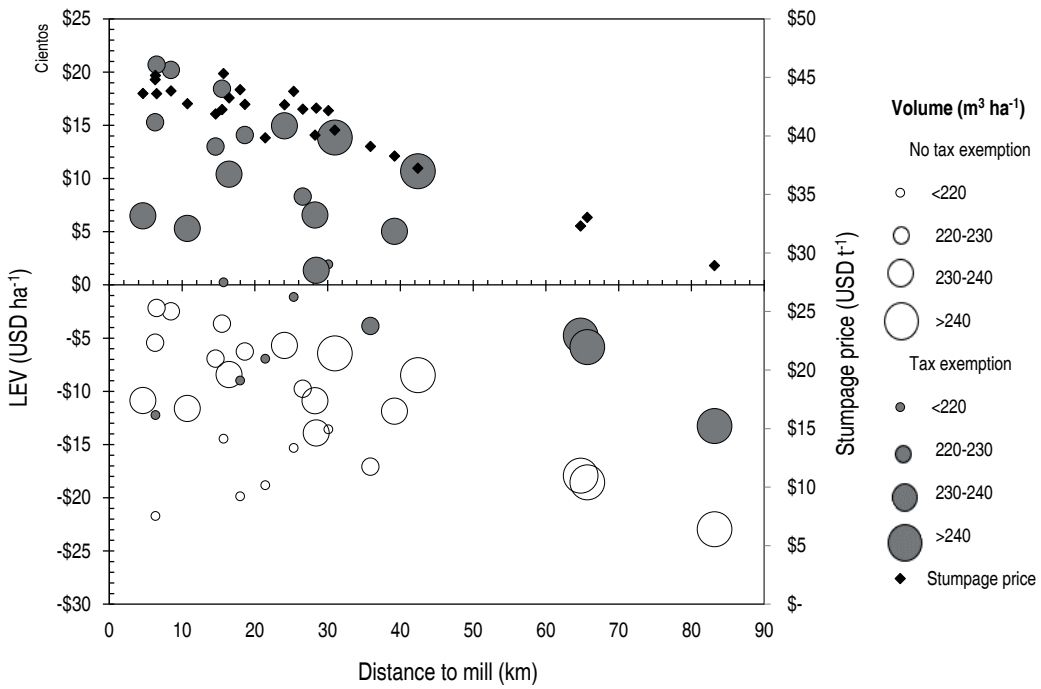

Figure 6. Relationship between the distance to the mill, volume, stumpage price, and Land Expectation Value (LEV).

New scenarios were evaluated considering the income tax exemption (33%) and simultaneous changes in costs and incomes. It was found that in the Northern Plateau, Barbosa, and Eastern Antioquia, average positive LEVs can be obtained without a decrease in costs or an increase in incomes. Tracts located around Northern Plateau and Barbosa can be classified as the most suitable areas for establishing new forest plantations in the region. The

Eastern Antioquia is also a promising geographical area in terms of the average LEV. However, higher variability of the LEV was observed in this region.

In all of the evaluated scenarios, the LEV was more sensitive to changes in costs than incomes (represented either on a stumpage price or a volume increase). The original theoretical representation of the LEV (Amacher *et al.*, 2009), can be used to explain why changes in costs affect the LEV to a greater extent than changes in incomes:

$$LEV_0 = \frac{PVe^{-rt} - C}{1 - e^{-rt}} \qquad (8)$$

Where LEV_0 is the LEV without modification in cost or income, P is the timber price, V is the total stand volume (yield), r is the continuously compounded return rate, t is the rotation age, and C is the costs of establishment and management (lump-sum). If the income is increased by a factor of α, LEV can be written as:

$$LEV_1 = \frac{\alpha PVe^{-rt} - C}{1 - e^{-rt}}, \qquad (9)$$

The relative change of the LEV (Δ_p), expressed as Equation 9 minus Equation 8, and expressed in relative terms of Equation 8, can be written as:

$$\Delta_p = \frac{(LEV_0 - LEV_1)}{LEV_0} = \frac{PV(1-\alpha)}{\boldsymbol{PV - Ce^{rt}}} \qquad (10)$$

If we define $LEV_2 = \frac{PVe^{-rt} - \alpha C}{1 - e^{-rt}}$, and calculate a similar relative change for the costs (Δ_c), the following expression is obtained:

$$\Delta_p = \frac{(LEV_0 - LEV_2)}{LEV_0} = \frac{Ce^{rt}(1-\alpha)}{\boldsymbol{PV - Ce^{rt}}} \qquad (11)$$

The term $(1-\alpha)/(\mathbf{PV}-\mathbf{C}e^{rt})$ appears in Equations 10 and 11, therefore, the potential effect of changes in costs or incomes is determined by analyzing how different PV and Ce^{rt} are. If these factors were estimated for the tracts in the study area at the rotation age (which implies the estimation of the future value of all the costs, C), costs are lower than the present value of the incomes. Nevertheless, the factor PV is less than 1.5 C and given that the factor e^{rt} varies in the range 2.4-3.8, for the rate of return (r=6.8%) and the optimal rotation age (t=T) found in this study (13–15 years), the factor Ce^{rt} will be always higher than the factor PV. Therefore, it can be concluded that variations in costs (Δ_c) have a bigger influence on the LEV than variations in incomes (Δ_p).

This lower rotation age (13-15 years) was found to be the main cause of the differential effects of variations in costs and incomes on the LEV. These values are nonetheless consistent with the optimal rotation age found by Restrepo *et al.* (2012) for *P. patula* in Colombia, varying from 12 to 14 years.

Equations 10 and 11 can also be used to explain how changes in the costs or incomes can differently affect the LEV in the study area. From these equations, it can be seen that Δ_p and Δ_c are a function of P, V, C and t. For simplicity, if it is assumed that the factor PV is constant, Δ_p will be a function with a vertical asymptote at $t_\alpha = ln\left(\frac{PV}{C}\right) \times \frac{1}{r}$. It was found that Δ_p will differ for each rotation age according to its location relative to the asymptote t_α. If $t > t_\alpha$, Δ_p decreases exponentially as t increases. If $t < t_\alpha$, Δ_p increases exponentially as t increases. As explained before, the relation PV/C is lower than 1.5 for all the tracts, leading to values for t_α lower than 6. Since $t > t_\alpha$ (t is between 13-15 and t_α is <6), the magnitude of Δ_p decreases as the rotation age increases.

Figure 7 indicates that tracts with higher optimal rotation age(i.e., tracts with lower incomes due to the low productivity, lower stumpage price, or a combination of both), are less sensitive to changes in incomes (lower Δ_p). On the contrary, those tracts with higher incomes (lower rotation age), are more sensitive to changes in the incomes (higher Δ_p). Considering a non-constant increasing factor PV would lead to increases in Δ_p. Figure 7 shows both the effect of the rotation age and the factor PV on the LEV change after applying a 1.1 factor increase in incomes. The same conclusion can be drawn for changes in costs. Similar asymmetry in the LEV was observed by Restrepo and Orrego (2015) for teak plantations in Colombia. The confidence interval estimated for the LEV in their study was considerably wider when lower rotation ages were considered. The

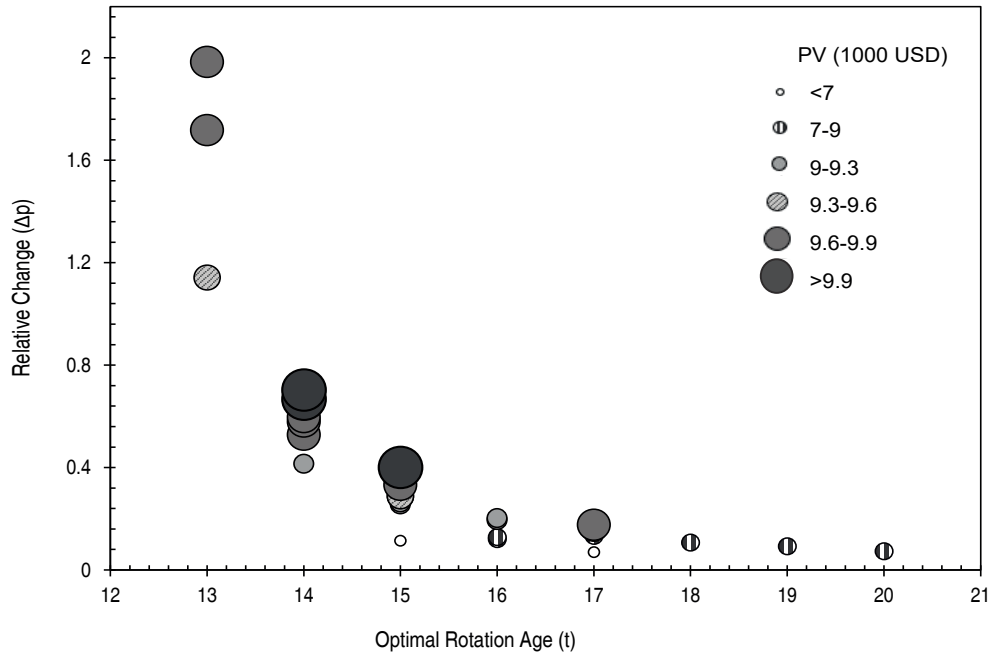

Figure 7. Effect of the optimal rotation age and income factor (PV) on relative change in incomes (Δ_p)

previous analysis showed that from an investment perspective, it is crucial to determine how sensitive the investment is to possible changes in factors such as the establishment costs, timber price, and volume. In general, for highly productive sites, it is more critical to determine the variations accurately in incomes and costs since this potential variability will have substantial effects on the profitability of the timberland investment. For those sites with either low productivity or low timber prices, the analysis of variations in incomes and costs are not expected to be relevant compared to more productive sites.

According to the results from this research, the priority areas for the establishment of new *P. patula* commercial plantations are located around the Northern Plateau and the Eastern Antioquia. Nonetheless, other factors can influence the decision to establish new forest plantations, such as the opportunity cost of the land reflected in the land price. According to Niskanen (1998), and from an economic perspective, forest plantations should be established on sites with a low opportunity cost since higher yield rates might not compensate for the increase of the opportunity cost of more productive land. This is relevant for the area around Eastern Antioquia, where urban development and physical expansion of the city of Medellín are already occurring (López *et al.*, 2010).

CONCLUSIONS

Site selection studies for establishing new commercial plantations should integrate both biophysical and financial analyses. Biophysical analyses are critical to identifying the spatial variability of the local productivity, but instead of being the final decision criterion, these studies are the starting point to perform a comprehensive analysis that includes financial analysis. This analysis allows for the identification of profit variability as well as the assessment of the critical factors that affect income, and ultimately influence the site selection from an investor perspective.

It was found for *P. patula* in Antioquia that under the current conditions of costs, productivity, and stumpage price, a positive LEV cannot be obtained unless government subsidies are provided. An MAI higher than 16 m^3 ha^{-1} $year^{-1}$, a distance less than 45 km to the mill, and a stumpage price higher than 35 USD t^{-1}, seem to be critical determinants for a profitable timberland investment. A government incentive, in the form of the income tax exemption, was found to be critical for the

profitability of potential timberland investments. Without this subsidy, a reduction of 20% in costs or an increase in productivity of the same proportion, would be required to obtain IRRs higher than a discount rate of 6.8%.

The sensitivity analysis suggests that a reduction in costs has a higher effect on profitability than an equivalent increase in incomes. A significant proportion of these costs originates from transportation due to the topographical characteristic of mountainous regions of Antioquia, where the government poorly maintains roads. An alternative will be to promote scale economies by consolidating the entire study area as a cluster, with a potential annual production of ~1,773,376 m^3 of roundwood. Another alternative will be to increase incomes through silvicultural activities, which can lead to a higher proportion of roundwood large size timber (higher stumpage price). Activities such as fertilization, vegetation control, and thinning are proposed to increase both round wood size and quality. Nevertheless, individual cost analyses should be done to evaluate the trade-off between costs and corresponding incomes. Having an integrated industry that elaborates the end-user product such as laminated boards or paperboard products (as it is the case of the two biggest forest products companies in Colombia), can also increase the profitability by increasing incomes and reducing unitary costs.

The methodology applied allowed for the estimation of the spatial variability of potential profits of *P. patula* in Antioquia. Relatively little information was required to generate the stumpage price map and the volume estimation. The availability of growth models using environmental covariates, along with additional information about transportation costs and timber price, would allow for the replication of this analysis on a broader scale and for different species, which would serve as a guide for future timberland investments in Colombia.

Uncertainty was included through the sensitivity analysis applied to variations in costs and incomes. Long-term time series of the economic variables such as discount rate, timber price, and transportation costs, would be required to complement our risk analysis. Dynamic studies that consider a spatial and temporal variation of these variables are also suggested. Nonetheless, this information is not currently available in Colombia since data associated with commercial forest operation is scarce and collected informally.

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Appendix 1.

Table A1. Detailed costs used in the financial analysis. all the values are in USD ha^{-1}.

	Consumables	Year 0	Year 1	Year 2	Year 3	Year 4	Year 5
	Direct Cost						
Workforce	Land preparation	92.0	0.0	0.0	0.0	0.0	0.0
	Marking	73.6	0.0	0.0	0.0	0.0	0.0
	Hole digging	183.9	0.0	0.0	0.0	0.0	0.0
	Seedlings transportation	18.4	0.0	0.0	0.0	0.0	0.0
	Planting	73.6	0.0	0.0	0.0	0.0	0.0
	phytosanitary control	9.2	18.4	18.4	18.4	18.4	18.4
	Fertilizers applications	55.2	64.4	0.0	0.0	0.0	0.0
	Replanting	36.8	0.0	0.0	0.0	0.0	0.0
	Vegetation clearing	183.9	183.9	183.9	92.0	0.0	0.0
	Forest roads maintenance	9.2	9.2	9.2	9.2	9.2	9.2
	Fire protection	92.0	0.0	0.0	0.0	0.0	0.0
	SUBTOTAL	827.7	275.9	211.5	119.5	27.6	27.6
Supplies	Seedlings	187.2	0.0	0.0	0.0	0.0	0.0
	NPK fertilizer	50.3	61.9	82.5	0.0	0.0	0.0
	Ant control	3.0	3.0	3.0	3.0	3.0	0.0
	Borax	11.6	0.0	14.2	0.0	0.0	0.0
	Animal feeding	2.1	0.0	0.0	0.0	0.0	0.0
	SUBTOTAL	254.1	64.9	99.7	3.0	3.0	0.0
	TOTAL	1,081.8	340.8	311.2	122.6	30.6	27.6
	Indirect Costs						
	PPE	41.4	13.8	10.6	6.0	1.4	1.4
	Supplies transport	38.1	9.7	15.0	0.5	0.5	0.0
	Technical assistance	54.0	54.0	54.0	54.0	27.0	27.0
	TOTAL	133.5	77.5	79.5	60.4	28.8	28.4
	TOTAL COSTS	1,215.2	418.3	390.7	183.0	59.4	55.9
	Expenses						
	Administration fees	89.1	28.6	24.8	10.9	2.7	2.5
	TOTAL EXPENSES	89.1	28.6	24.8	10.9	2.7	2.5
	TOTAL COSTS+EXPENSES	1,307.1	446.9	415.5	193.9	62.0	58.4

ÍNDICE DE AUTORES

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

La Revista Facultad Nacional de Agronomía Medellín (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 Revista recibe y publica, sin ningún costo, artículos en idioma inglés de investigación, revisión, reseñas, cartas al editor y editoriales.

La periodicidad de la Revista es cuatrimestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales, inéditos y arbitrados (peer review) 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. La extensión máxima debe ser de 5200 palabras, excluyendo figuras, tablas, referencias. El mínimo de referencias bibliográficas sugerido es de 10 y el máximo de 30. Este tipo de artículos es arbitrado e indexado.

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. La extensión máxima debe ser de 6000 palabras, excluyendo figuras, tablas, referencias. Este tipo de artículos es arbitrado e indexado.

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.

El autor(es) se compromete(n) a ceder los derechos de impresión y reimpresión del material publicado a la Revista Facultad Nacional de Agronomía y cualquier cita a los artículos editados en la Revista se deberá hacer si se adiciona el crédito respectivo. En caso de duplicación del contenido de la Revista o su publicación parcial o total en otro idioma, se deberá contar con el permiso escrito del Director.

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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 deben remitir los siguientes cuatro formatos: (1) Lista de verificación de criterios editoriales para la presentación de manuscritos; (2) Autorización de publicación de manuscritos en la Revista Facultad Nacional de Agronomía Medellín, en la 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, este debe ser firmado por todos los autores del manuscrito; (3) Datos personales de cada autor; (4) Sugerencia de posibles pares evaluadores. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión 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 Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. El manuscrito no debe exceder 5200 palabras para artículos de investigación y 6000 para artículos de revisión. Las hojas deben ser tamaño carta, escritas a interlineado doble, numeración de línea continua, 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 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. Las unidades combinadas deben 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 título de pregrado, el cargo laboral de los autores, 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 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

Puede tener o no título. 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

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) 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. Las tablas se deben elaborar con pocas columnas y renglones. 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.

Formato de citación en el texto

- Se registra la fuente entre paréntesis, el cual debe incluir el apellido del autor y año, con coma entre autor y año. Ejemplo: (Pérez, 1995).

- Si hay más de una fecha se separarán con comas: Ejemplo: (Pérez, 1995, 1998, 2001)

- Si hay dos autores se citarán separados por la conjunción and. Ejemplo: (Gil and 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.* (en cursiva) que significa y otros; en la referencia se deben poner los apellidos e iniciales de todos los autores. Ejemplo: (García *et al*., 2004).

- Cuando se hace referencia al autor dentro del texto, sólo se encierra el año entre paréntesis y se omite la coma que separa al autor del año. Ejemplo: (1) De acuerdo con Castañeda (2000), …; (2) Concorde con los resultados de Poveda *et al.* (2018) …

- Cuando es una cita de una cita se ponen la información de los autores citados y los autores citantes. Ejemplo: Magalhaes *et al.* (1979) expone que … (as cited in Gómez, 2004).

- Organizaciones se citan por sus siglas, en caso de no tener se cita con su nombre completo. Ejemplo: (1) (FAO, 2015), (2) (Ministerio de Agricultura y Ganadería, 2019)

Referencias

Sólo se listan las referencias bibliográficas mencionadas en el texto. No se aceptan notas de clase o artículos en preparación, o cualquier otra publicación de circulación limitada.

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, se 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 publicación.

En cada referencia para todos los autores cite primero el apellido, tener en cuenta que algunos autores hispanos citan sus dos apellidos, seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Libros: Autor(es). Año. Título del libro. Edición. Casa editora, ciudad de su sede. 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.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

Capítulos de libros: Autor(es). Año. Título del capítulo. Páginas consultadas (pp. # - #). En: Apellidos e iniciales de los compiladores o editores (eds.). Título del libro. Edición. Casa editora, 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.

Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Artículos de revistas: Autor(es). Año. Título del artículo. Nombre completo de la revista volumen(número de fascículo): 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

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High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

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Adam M. 1992. The Impact of the Common Agricultural Policy on Agriculture in Greece (Master's thesis). Cambridge University. Cambridge, United Kingdom. 80 p.

Cita de cita, sólo se referencia la fuente consultada. Ejemplo: Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia.

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 Suppl. 2: 195-201.

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. In: Agricultura Tropical, http://agrotropical.edunet.es. 25 p. consulta: noviembre 2003.

Patentes: Autor(es). Año. Título. País de la patente y número. Fuente. Ejemplo: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA MEDELLÍN

The Journal *Revista Facultad Nacional de Agronomía Medellín* (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at professors, 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 receives and publishes, without any cost, research articles, reviews, revisions, letters to the editor and editorials written in the English language.

The Journal is a four-monthly publication at national and international level. Its aim is to publish original, unpublished, and peer-reviewed 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.

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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. The maximum extension must be 5200 words; excluding figures, tables, references. The maximum number of bibliographic references suggested is 30. This type of article is peer-reviewed and indexed.

Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references. The maximum length must be 6000 words; excluding figures, tables, references. This type of articles is arbitrated and indexed.

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INSTRUCTIONS TO AUTHORS

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Papers must be sent b 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. The four following formats must be submitted with the manuscript: (1) Editorial Criteria Checklist for Paper Submission; (2) Paper Publishing Authorization for the Revista Facultad Nacional de Agronomía Medellín, which accepts no simultaneous nomination of the article to other journals or editorial bodies, and the rights are given to the Journal for its release by the signature of all the manuscript's authors; (3) Personal information of each author; (4) Suggestion of possible peer reviewers. 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 for research articles and 6,000 words for reviews. The manuscript must be letter-size sheets, line spacing double, continuous line number 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 2002 to 2005, growing seasons 1999-2000, 2000-2001.

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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 and Spanish. 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.

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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.

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It refers to the analysis and objective interpretation of results, comparing them with those obtained in other research, 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.

Citing in-text format

- Citations in the text should be in parenthesis and include author's surname and year, with comma in-between. Example: (Pérez, 1995).

- 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 of 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 author and replace the others by the Latin expression *et al.* (in italics), which means and others. All authors should be mentioned in the reference. Example: (García *et al.*, 2004)

- When the author is referenced within the text, only the year is enclosed in parentheses, and the comma that separates the author from the year is omitted. Example: (1) According to Castañeda (2000), ...; (2) In accordance with the results of Poveda *et al.* (2018), ...

- When an indirect source is cited, the information of the cited authors and the citing authors are placed. Example: (Magalhaes *et al.* (1979) state that ... (as cited in Gómez, 2004).

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References

Only bibliographical references cited in-text are listed in the references section. Lecture notes, articles in preparation, or any other publication with limited circulation are not accepted. Excessive self-citation should be avoided.

Bibliographic references are ordered alphabetically by first author's surname, without numbering and without indentation. To cite several publications of the same author, chronological increasing order must be followed. Alphabetical order of titles must be followed in case they are from the same year.

References should contain all the data allowing to its easy location. The titles of the papers, the surnames of the authors and the names of journals must be referenced and cited in their original language.

Examples:

For books: Author(s), Year. Book title, Edition, Publisher, Place of publication. Pages consulted (pp. #-#) or total pages.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.

García Rodríguez JL, Giménez Suarez MC, Ortega Pérez E, Martín Ramos B, Calderón Guerrero C. 2014. Operaciones auxiliares en repoblaciones e infraestructuras forestales. Ediciones Paraninfo SA, Madrid. 208 p.

For book chapters: Author(s). year. Chapter title. pages consulted (pp. # - #). In: Surnames and names of the editors or publishers (eds.). book title. Edition. Publisher, place of publication. total pages (# p.). Example: Bertoft E and Blennow A. 2016. Chapter 3 - Structure of potato starch. pp 57-73. In: Singh J and Kaur L. (eds.). Advances in potato chemistry and technology. Second edition. Academic Press, London. 752 p.

Beral H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

For journals: Author(s). year. Article title. journal full name volume(number): initial page-final 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. 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, Bogotá.

High R. 2015. Plotting LSMEANS and Differences in Generalized Linear Models with GTL. In: 2015 Midwest SAS Users Group Conference Proceedings. Midwest SAS Users Group, Omaha. 9 p.

Theses and dissertations: Adam M. 1992. The impact of the common agricultural policy on agriculture in Greece (Doctoral dissertation). Cambridge University. Cambridge, United Kingdom. 80 p.

Gómez C. 2004. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín. Colombia. 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.

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Patents: Author(s). Year. Title. Patent country and number. Retrieved from. Example: Glenn RW. 1996. Liquid personal cleansing compositions which contain soluble oils and soluble synthetic surfactants. U.S. Patent No. 6194364. Retrieved from: https://patents.google.com/patent/US6194364B1/en

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.

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3.1. Hace referencia a la adición, supresión o reorganización de los nombres de autor en la autoría de un artículo aceptado.

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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]

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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.

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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.

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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.
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5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
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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.
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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:

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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.
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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.
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8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
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8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
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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.

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[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.

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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.

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