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EVALUADORES

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

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EDITORIAL

Ochenta años de la Revista Facultad Nacional de Agronomía Medellín 1939 – 2019

Cuando nace la Revista

Hace ocho décadas nació la Revista Facultad Nacional de Agronomía Medellín, al calor del vigésimo quinto aniversario de la Facultad Nacional de Agronomía y del primer Congreso de Ingenieros Agrónomos de Colombia.

La Revista aparece en el contexto de las reformas educativas de 1935, en virtud de las cuales el Instituto Agrícola Nacional termina convertido en Facultad, gracias a la intensa labor del Dr. Jorge Gutiérrez Escobar quien era el director del Instituto y al Dr. Francisco Luis Gallego, Entomólogo, quienes logran que el Instituto sea anexado a la Universidad Nacional de Colombia en diciembre de 1937; mediante el Acuerdo 103 del 30 de noviembre de 1938, la Universidad Nacional de Colombia erige al Instituto como Facultad Nacional de Agronomía, que pasa a conformar el nombre de la Revista.

Siendo ya Decano de la naciente Facultad, el Dr. Jorge Gutiérrez Escobar, en unión con los directores de la Revista, profesores, José V. Lafaurie Acosta y Jesús Atehortúa Ramírez, publican la primera edición, Año 1 agosto de 1939 Vol. I - No. 1. Estos emprendedores tenían claro que el lanzamiento de la Revista era un desafío financiero y humano, y sobre todo, sabían que era una gran aventura intelectual, de la cual ya han transcurrido 80 años de publicación ininterrumpida. En sus inicios, los patrocinadores fueron: Coltejer, Fabricato, Tejicondor, Trapiches Amagá, Tabaco Piel Roja, Algodonera Colombiana S.A., Federación Nacional de Cafeteros, entre otras agroindustrias florecientes en Antioquia. Luego se establecieron pautas comerciales y un pequeño aporte para quienes publicaban artículos en ella. De un tiempo para acá, se financia a través de la decanatura y de los fondos de investigación y extensión.

Objetivos de la Revista

Uno de los objetivos principales e iniciales con los que nace la Revista, fue crear una “Cátedra libre”, disponiéndose con ella, a convocar a los estudiosos del sector agrario y de la realidad nacional, a enriquecer sus páginas con el debate y la investigación.

En adición se proyectó la divulgación de trabajos científicos, la “redención” del campo y del campesino, la difusión entre los productores agrarios de los conocimientos necesarios para explotar técnica y científicamente la tierra, la creación de una conciencia nacional sobre la necesidad de una intervención proteccionista para el sector agrario incluido el forestal, la publicación de tesis de grado y la defensa de los Profesionales Agrarios.

En sus inicios era el órgano de divulgación de la Facultad Nacional de Agronomía en la cual publicaban los docentes, investigadores y estudiantes de la Facultad, trabajos de tesis e investigaciones y monografías temáticas. En 1971 tuvo el primer cambio a Revista de divulgación Científica y Académica, publicando artículos de investigación bajo la estructura y lineamientos científicos. En 1974 ya es registrada como una publicación periódica y se le asigna el ISSN, indicación de calidad editorial.

Inicialmente no tenía una periodicidad definida y a partir de 1989 se publica 2 veces al año, y en 2017 la periodicidad de la edición cambia a cuatrimestral, tres números por volumen.

Admiración y Reconocimiento

Destacados investigadores y docentes han manifestado su admiración y reconocimiento por la calidad de su contenido y presentación; equiparándolo con las mejores publicaciones de las comunidades científicas y académicas del mundo.

En esos términos se han referido varios egresados de la Facultad de Ciencias Agraria de la Universidad Nacional de Colombia, entre los que se pueden mencionar a: Jairo Cano Gallego, Ph.D, exinvestigador del CIAT (Centro Internacional de Agricultura Tropical), exfuncionario del IICA y ex asesor del Ministerio de Agricultura en Colombia. En términos similares se han referido

a ella, el Fisiólogo y exprofesor de la Universidad Nacional de Colombia, Jairo Correa Velásquez, Ph.D; Luis Hernán Rincón Rincón, Ph.D, quien trabajó en la Universidad EARTH de Costa Rica y en la Universidad Agraria La Molina del Perú. Cesar Mendoza de Armas Ingeniero Agrónomo de la Universidad Central de Venezuela, Ph.D de la Sorbona Paris I, afirma "Es importante señalar, que la forma en que son elaborados, diseñado y publicados los trabajos que forman parte de la Revista, permite a los lectores entender el desarrollo de las investigaciones y evaluar los resultados obtenidos, de una forma precisa". Dice además que "En Francia, Israel, Venezuela, Canadá, México y España, donde se le da gran importancia a lo agrario, es difícil conseguir una publicación de la Calidad de la Revista Facultad Nacional de Agronomía Medellín". El profesor Diego Alonso Restrepo Molina, exdirector de la Revista, manifestó que sintió palpitación cuando la encontró en bibliotecas de Israel y Egipto.

La perseverancia de sus directores/orientadores y del comité editorial, el apoyo de los respectivos decanos, las exigencias y rigurosidad de los editores técnicos y pares evaluadores en cuanto a su exhaustiva calidad, la han mantenido sucesivamente en el tiempo como publicación científica y académica. Estas exigencias, afirma su directora actual Edith Marleny Cadena Chamorro, Ph.D, son la mejor herramienta para hacer transferencia científica a nivel mundial.

En esta forma, se está compitiendo con Revistas a nivel local, nacional e internacional; sin duda alguna, es un inicio para avanzar a futuro en la búsqueda de un mejor posicionamiento. Después de que su carácter divulgativo cambia, apostándole más a lo científico, y que su presentación es en el idioma inglés; la Revista gana visibilidad e impacto, gracias a la indexación en "base de datos" internacionales y al canje nacional e internacional con importantes bibliotecas universitarias y con instituciones científicas del mundo agrario. Las Revistas indexadas son publicaciones periódicas de investigación que denotan alta calidad y que han sido listadas en publicaciones de investigación de consulta mundial, lo que habitualmente trae consigo que tengan un elevado factor de impacto.

Actualmente, se publican artículos bajo lineamientos estrictos de Colciencias, el porcentaje de autores de la propia institución editora es igual o menor al 50%. La revista ha sido atractiva para autores internacionales, se destaca la participación de países como Brasil, Ecuador, México, Perú, España, Nigeria, Cuba, Argelia, entre otros. La edición incluye todos los ejes del sector agrario: Ciencias Agronómicas, Producción Animal, Ciencias Forestales, Ingeniería Agrícola y de Alimentos, y otras áreas multidisciplinarias que comprenden el área ambiental, biotecnología, producción limpia, etc, temáticas que contribuyan a la solución de los limitantes del agro.

Hoy, a los 80 años de existencia de la Revista, su directora, opina que su permanencia como publicación periódica científica y académica, debe continuar fortaleciéndose acorde a los criterios de calidad y política editorial, continuar trabajando según los estándares nacionales e internacionales de comunicación científica y continuar con el compromiso institucional de transferencia tecnológica de acceso abierto al conocimiento en Ciencias Agrarias.

En el marco de los 80 años de la Revista Facultad Nacional de Agronomía Medellín, destacamos la labor de sus directores(as), cuyo compromiso y dedicación, a lo largo de estos años, ha enriquecido la divulgación científica y académica de las Ciencias Agrarias.

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A ellos y a todos los que han sido participes de tan valiosa labor, muchas gracias.

Gustavo Jiménez Narváez
Coordinador Oficina de Egresados
Facultad de Ciencias Agrarias
Universidad Nacional de Colombia Sede Medellín.

Research article

http://www.revistas.unal.edu.co/index.php/refame

Revista Facultad Nacional de Agronomía

Dependency, colonization, and growth in *Gmelina arborea* inoculated with five strains of Arbuscular Mycorrhizal Fungi

UNIVERSIDAD NACIONAL DE COLOMBIA

Dependencia, colonización y crecimiento en *Gmelina arborea* inoculada con cinco cepas de Hongos Micorrízicos Arbusculares

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Keywords:
Commercial forests
P fertilization
Rhizoglomus spp.

ABSTRACT

Gmelina arborea is a forest species of increasing use in the establishment of commercial plantations in Colombia. The areas where it is currently planted are deficient in nutrients, so the use of Arbuscular Mycorrhizal Fungi (AMF) can be an alternative to improve phosphoric fertilization. The aim of this work was to determine the mycorrhizal dependency, colonization, and growth of *G. arborea* when it is inoculated with *Rhizoglomus fasciculatum, Rhizoglomus aggregatum, Rhizoglomus irregulare, Glomus fstulosum*, and *Entrophospora colombiana*, under different concentrations of phosphorus (P) in a soil solution. A completely randomized design was used with a 6×3 factorial arrangement, five AFM strains + control (uninoculated) and three P doses (0.002, 0.02, and 0.2 mg L^{-1}) with five replicates per each treatment and twice through time. Mycorrhizal colonization and dependency, foliar concentration of P, dry biomass, leaf area, and height were evaluated. A moderate mycorrhizal dependency was obtained under a P concentration of 0.002 and 0.02 mg L^{-1} and inoculation with *R. fasciculatum*, *R. aggregatum*, and *R. irregulare* while inoculation with *G. fstulosum* and *E. colombiana* produced a marginal dependency. It was found a negative effect on *G. arborea* inoculated with all AMF strains under 0.2 mg L^{-1} of P. Mycorrhizal colonization presented values between 62.5 - 2.5% for all the AMF evaluated, influenced by AFM strains and P concentration. Plants inoculated with *R. fasciculatum*, *R. aggregatum,* and *R. irregulare* showed a signifcant increase ($P<0.05$) in their growth. Mycorrhizal dependency and colonization in *G. arborea* and its growth were highly influenced by species of AMF and amount of P.

Palabras clave:
Bosques comerciales
Fertilización P
Rhizoglomus spp.

RESUMEN

Gmelina arborea es una especie forestal de uso creciente en el establecimiento de plantaciones comerciales en Colombia. Las zonas donde es actualmente plantada son deficientes en nutrientes, por lo que el uso de Hongos Micorrízicos Arbusculares (HMA) puede ser una alternativa para mejorar la fertilización fosfórica. El objetivo de este trabajo fue determinar la dependencia y colonización micorrizal, el crecimiento de *G. arborea* inoculada con *Rhizoglomus fasciculatum*, *Rhizoglomus aggregatum*, *Rhizoglomus irregulare*, *Glomus fistulosum* y *Entrophospora colombiana*, bajo diferentes concentraciones de fósforo (P) en la solución de suelo. Se utilizó un diseño completamente al azar, con un arreglo factorial 6×3, cinco cepas de HMA + un control (sin inocular) y tres dosis de P (0,002, 0,02 y 0,2 mg L^{-1}), con cinco replicas por tratamiento y dos a través del tiempo. La colonización y dependencia micorrizal, la concentración foliar de P, la biomasa seca, el área foliar y la altura fueron evaluadas. Una dependencia micorrizal moderada se obtuvo bajo una concentración de P de 0,002 y 0,02 mg L^{-1} y la inoculación con *R. fasciculatum, R. aggregatum y R. irregulare* mientras que la inoculación con *G. fistulosum* y *E. colombiana* produjo una dependencia marginal. Se encontró un efecto negativo en *G. arborea* inoculada con los HMA bajo una concentración de 0,2 mg L^{-1} de P. La colonización micorrizal presentó valores entre 62,5 - 2,5% para todos los HMA evaluados, influenciados por las cepas de HMA y el nivel de P. Las plantas inoculadas con *R. fasciculatum, R. aggregatum* y *R. irregulare*, mostraron un aumento significativo ($P<0.05$) en su crecimiento. La dependencia y la colonización micorrizal en *G. arborea* y su crecimiento estuvo altamente influenciado por las especies de HMA y la cantidad de P.

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Gmelina arborea Roxb. ex Sm is one of the most promising forest species known worldwide in the use of different reforestation programs due to its rapid growth, it is a secure source of raw wood in a short time. This plant behaves as an opportunistic in rainforests, and it is classified as a pioneer (Moya, 2004; Rojas *et al*., 2004; Hernández and Salas, 2008). This species is currently being used in commercial forest plantations, and it is commonly used on the Atlantic coast with around 11,000 planted hectares in Colombia (Cadena and Guauque 2009; Romero, 2004). The growing importance that this species is having is due to relative homogeneity and stability of its wood which makes it adequate for obtaining cellulose as raw material for paper making (Dvorak, 2004). Other uses are furniture manufacturing (Moya, 2004; Romero, 2004), living fences, windbreaker curtains and timber boundaries (Rojas *et al*., 2004).

Gmelina arborea is a perennial plant, which makes it a key step in nursery seedling production that determines the success of the future plantation, making necessary to look for alternatives that improve the development and fertilization at this stage of the production cycle. This is how the use of Arbuscular Mycorrhizal Fungi (AMF) becomes a viable option to fulfill this objective because of these microorganisms support the growth, survival and seedling development in different vegetable species under tropical conditions (Osorio, 2011; Ramírez-Gil *et al*., 2013; Ramírez-Gil *et al*., 2014; Ramírez-Gil *et al*., 2015).

AMF improves the growth of its host, especially in soils with low levels of phosphorus and under stress due to lack of water (Yano and Takaki, 2005; Osorio, 2011; Osorio *et al*., 2017). This symbiosis also helps the absorption of other nutrients, improves the response to the attack of pathogenic microorganisms, among other benefits (Osorio, 2011; Ramírez-Gil *et al*., 2014; Osorio *et al*., 2017). It is expected that inoculation with AMF in *G. arborea* improves the productivity of this species in Colombia since most areas planted are arid zones and have soils with low natural fertility (Cadena and Guauque, 2009). Besides they are an economical and friendly alternative to protect the environment by reducing fertilizer applications on soil, especially with phosphorus (P) fertilizers (Osorio, 2011; Osorio *et al*., 2017).

The first step for an adequate use of AMF in agriculture consists in determining the relationship between microorganism strains with the host species, which can be quantified through mycorrhizal colonization and dependency (Osorio, 2011; Ramírez-Gil *et al*., 2013; Ramírez-Gil *et al*., 2014; Ramírez-Gil *et al*., 2015; Osorio *et al*., 2017). Given the need to understand the symbiotic relationship between *G. arborea* and different AMF species, this work aimed to determine the mycorrhizal dependency and colonization, so as the plant development when was inoculated with five AMF species (*R. fasciculatum, R. aggregatum, R. irregulares, G. fistulosum,* and *E. colombiana*) in three different P levels in a soil solution (0.002, 0.02, and 0.2 mg L^{-1}).

MATERIAL AND METHODS

Location

The experiment was performed between 2012 and 2013, in the Environmental Microbiology and Tropical Phytotecnia laboratories and greenhouse at the Universidad Nacional de Colombia, in Medellín (6°15'51.7"N, 75°34'40.1"W, and 1495 m of altitude). The greenhouse environmental conditions were: temperature at 18-22 °C, relative humidity in a range of 75-95%, and photosynthetically active radiation of 650-1920 µmol photons m^{-2} s^{-1}.

Obtaining soil and seedlings

The soil used in these assays was collected from the subsurface horizon (Bt) in Volador hill, Medellín, Antioquia, Colombia (6°15'56.4"N, 75°34'57.1"W). The soil sample was collected under 30-60 cm of deep to reduce the influence of native organic P available on the soil (Ramirez-Gil *et al*., 2015). The soil sample was analyzed based on protocols used in the Soil Fertility Laboratory at Universidad Nacional de Colombia-Sede Medellín. Soils results were: sand: 65%, silt: 15%, clay: 20% (Bouyoucos); pH 4.8 (water 1:2, v:v); Al: 0.7 $cmol_c$ kg^{-1} (KCl 1 M); Ca, Mg, K: 1.2, 0.5, 0.02 $cmol_c$ kg^{-1} (1 M ammonium acetate); Fe, Mn, Cu, Zn: 37, 3, 1, 2 mg kg^{-1} (Olsen-EDTA), B: 0.1 mg kg^{-1} (hot water); S: 9 mg kg^{-1} (0.008 M Calcium phosphate); NO_3^-: 2 mg kg^{-1} (0.025 M ammonium sulfate); NH_4^+: 2 mg kg^{-1} (KCl 1M); P: 2 mg kg^{-1} (Bray II); P soluble: 0.001 mg L^{-1} (0.01 M of $CaCl_2$.) and organic material: 2% (Walkley and Black method).

The soil was sterilized following the recommendations reported by Ramírez-Gil *et al*. (2015) to eliminate

microorganism interferences. Using an autoclave at 0.1 MPa and 121 °C, for two cycles of one hour each. Besides, the aim to improve the AMF ecological condition (Osorio *et al*., 2017), lime was added to adjust soil pH at 5.6, based on lime incubation method (Uchida and Hue, 2000). On the other hand, KH_2PO_4 was added to achieve a soil solution of P concentration of 0.002 0.02 and 0.2 mg L^{-1}, based on P sorption isotherm (Fox and Kamprath, 1970), which is considered optimal for the studied mycorrhizal dependence and activity (Habte and Manjunath, 1991; Osorio 2011; Osorio *et al*., 2017).

Seeds of *G. arborea* were collected from healthy trees in Santa Fe de Antioquia at the Cotove Agriculture Experimental Station (6°31'55.2"N, 75°49'33.8"W) of Universidad Nacional de Colombia Sede Medellín. The process of extraction, cleaning, disinfection, and germination was developed according to the indications reported by Ramírez-Gil (2017). The plants were maintained in autoclaved quartz until the experiment was assembled.

Obtaining microorganisms and their inoculation

The AMF strains used were *Rhizoglomus fasciculatum, Rhizoglomus aggregatum,* and *Rhizoglomus irregulare*. Originally provided by Dr. M. Habte from University of Hawaii (Honolulu, USA). They were subsequently multiplied following the indication of Environmental Microbiology Laboratory at the Universidad Nacional de Colombia Sede Medellín, using roots of sorghum plants. *Glomus fistulosum* and *Entrophospora colombiana* strains were supplied by a private laboratory and multiplied based on the indication reported before.

The inoculation in soil was developed based on methodology reported by Ramírez-Gil *et al*. (2015) using plastic pots containing 2 kg of the chosen soil. In each, 35 g of crude inoculum per kg of soil was applied. The inoculum presented a concentration of 45-50 infective mycorrhizal propagules per g of soil (Table 1), determined according to the most probable number method (Porter, 1979). In controls, an autoclaved (120 °C, 0.1 MPa, for 60 min) crude inoculum (35 g kg^{-1}) and 10 mL of a suspension (10%) obtained from inoculant filtered through a 10 µm filter paper were used (Ramírez-Gil *et al*., 2015). Then two seedlings were transplanted into plastic pots, a month later one of the plants was removed. The plants were kept for three months in net-house conditions under 50-60% of the soil's maximum water retention capacity. Every week 50 mL of a P-free Hoagland solution was added (Ramírez-Gil *et al*., 2015).

Table 1. Inoculum content of each Arbuscular Mycorrhizal Fungi (AMF) species used for inoculation of *Gmelina arborea.*

Species of AMF	Infective mycorrhizal propagules per g of soil
Rhizoglomus fasciculatum	45±1.5
Rhizoglomus aggregatum	48±1.8
Rhizoglomus irregulare	46±2.0
Glomus fistulosum	48±2.3
Entrophospora colombiana	50±2.5

± standard deviation.

Colonization and mycorrhizal dependence and development of *Gmelina arborea* to five species of AFM

A completely randomized design was used, with a factorial arrangement 6×3, five strains of AMF + control (uninoculated plans), and three doses of phosphorus in the soil solution, with five replicate per each treatment and twice through time. Experimental units consisted of three pots with one plant each.

Ninety days after the treatment started, biometrics and symbiotic variables were evaluated. The height (cm) was measured using a Mitutoyo Digimatic Caliper®, dry biomass (g) was quantified in by Binder® stove at 60 °C for 72 h, leaf area (cm^2) was measured using a LI-Cor® LI 3000A foliar area meter, and foliar phosphate concentration (%) was determined by non-destructive samples using the blue molybdate method (Murphy and Riley, 1962;

Aziz and Habte, 1987). Mycorrhizal Colonization (MC) was evaluated based on the following steps (i) roots were discolored using KOH (10%) for 24 h (Phillips and Hayman, 1970), (ii) coloration of roots by fuchsine acid staining (0.15%) for 48 h (Kormanik *et al*., 1980), (iii) excess of fuchsine on roots were removed using glycerin-lactic acid-water (0.7-0.2-0.1 v:v), and (iv) intensity of mycorrhizal colonization using the interception lines method (Giovannetti and Mosse, 1980). Mycorrhizal dependency (MD) was calculated based on total dry material value, using equation 1 proposed by Plenchette *et al.* (1983). MD classification was performed following the indications reported by Habte and Manjunath (1991), MD values of 75% or higher, 50-75%, 25-50%, less than 25% and 0% represents very highly, highly, moderately, marginally, and independent mycorrhizal dependency, respectively.

$$MD = \frac{Dry\ mass\ inoculated\ plants - Dry\ mass\ uninoculated\ plants}{Dry\ mass\ inoculated\ plants} x100 \quad (1)$$

Statistics analysis of data

For each variable evaluated, data homoscedasticity and normality were determined by Levene and Kolmogorov Smirnov methods. Once homoscedasticity and normality were proved, an ANOVA test was performed. Means were compared with the Tukey test (P<0.05). The evaluation was performed using the R software (R Core Team, 2013).

RESULTS AND DISCUSSION

Mycorrhizal colonization and dependency of *Gmelina arborea* with five AMF species

All plants of *G. arborea* inoculated with *R. fasciculatum*, *R. aggregatum*, *R. irregulare*, *G. fistulosum*, and *E. colombiana* presented mycorrhizal colonization, which was evidenced by the presence of hyphae in these fungi, particularly within intracellular spaces in the root. In uninoculated plants, no associated structures of AMF were found (Figure 1A and B).

Most mycorrhizal colonization (P<0.05) in *G. arborea* was found with inoculation of *R. fasciculatum* and *R. aggregatum* under 0.002 and 0.02 mg L^{-1} of P in the soil solution. These values were 62.5-55.5% and 55.2-54.5%, respectively. In decreasing order and without statistical differences within them (P>0.05), were found the inoculation *R. irregulare*, and *G. fistulosum* under 0.02 and 0.002 mg L^{-1} of P in the soil solution, with values of 38.3-36.2% and 35.3-34.8%, respectively. *E. colombiana* inoculation showed the lowest values (P<0.05) concerning other AMF species that were evaluated with a 22.3 and 26.2% of colonization under 0.02 and 0.002 mg L^{-1} of P in the soil solution, respectively. Also, the mycorrhizal colonization of these five AMF species under 0.2 mg L^{-1} of P did not exceed 5.9% (Figure 2A).

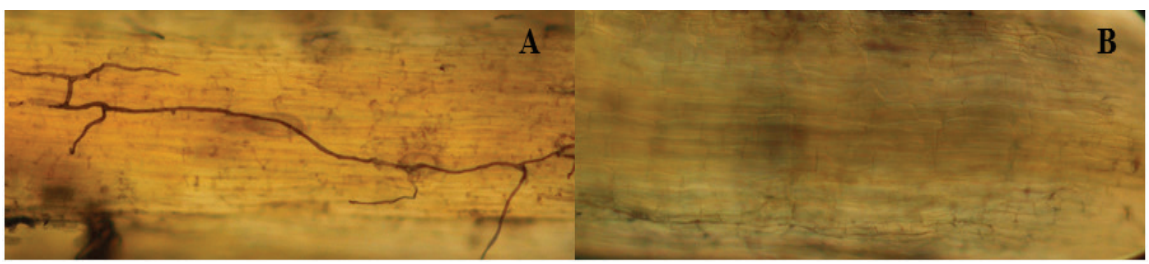

Figure 1. Presence and absence of arbuscular mycorrhizal fungi in roots of *Gmelina arborea*. A. root inoculated with an Arbuscular Mycorrhizal Fungi (AMF) (*Rhizoglomus fasciculatum*) (40x); B. root uninoculated (40x).

Based on Plenchete *et al.* (1983) formula (equation 1), and determined according to the classification criteria of Habte and Manjunath (1991), the mycorrhizal dependency (MD) in *G. arborea*, showed that this species presents a moderate dependency with respect to *R. fasciculatum*, *R. aggregatum*, and *R. irregulare* under P soil solution of 0.02 and 0.002 mg L^{-1} with values of 42.3-35.1%, 34.5-26.2%, and 28.3-26.2%, respectively (Figure 2B). Meanwhile,

G. fistulosum and *E. colombiana* MD was classified as marginally dependent for P levels of 0.02 and 0.002 mg L^{-1} with values of 14.8-15.3 and 10.2-12.3%, respectively. In this sense the *G. arborea* presented the highest MD to species of AMF *R. fasciculatum* (*P*<0.05) (Figure 2B). For the AMF species evaluated and with a P level of 0.2 mg L^{-1} in the soil solution, the MD values in *G. arborea* were negative, indicating that under these conditions of P in the soil the symbiotic relation becomes adverse for the plant (Figure 2B).

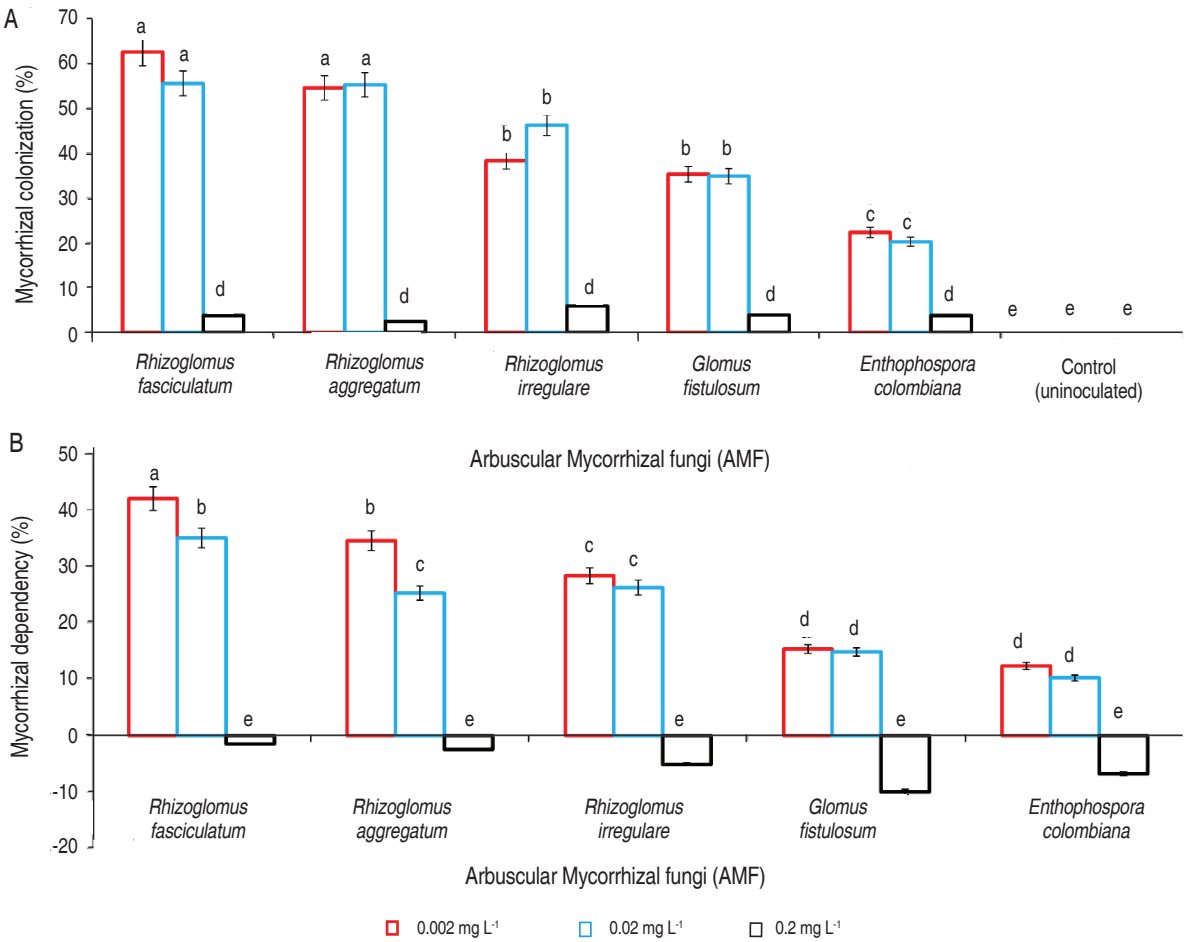

Figure 2. A. Mycorrhizal colonization; B. Mycorrhizal dependence in *Gmelina arborea* inoculated with five species of Arbuscular Mycorrhizal Fungi (AMF). P levels evaluated in the soil solution were 0.002, 0.02 and 0.2 mg L^{-1}. Statistical analyzes were performed for each level of P and each AMF species evaluated. Equal letters on the bars represent no significant differences with a significance level of 95%. Lines on the bars represent standard error of the mean.

The high MC and medium MD found in *G. arborea* show that this species needs positive symbiotic relations with AMF to achieve better development and survival, resulting in an improvement of the plant development under these circumstances. This relation was highly conditioned by the AMF species and the content of P in the soil solution, the best results were associated with P levels of 0.002 and 0.02 mg L^{-1} and *R. fasciculatum* inoculation, followed by other associated species of the *Glomus* genera. These results coincide with other works in different species, where inoculation with this same strain (*R. fasciculatum*) and under these soil conditions (P dose, 0.02 mg L^{-1}) presented high values of mycorrhizal colonization and dependency (Ramírez-Gil *et al*., 2013; Ramírez-Gil *et al*., 2014; Ramírez-Gil *et al*., 2015). According to Sieverding (1991), *Rhizoglomus*

is one of the most aggressive and effective generous for the establishment of symbiotic relations with different taxonomic plant groups under natural conditions.

The best development from symbiotic relation of *G. arborea* and the AMF species was found in a soil P dose of 0.02 mg L^{-1}. It takes place, thanks to the fact that under this condition the symbiotic relationship is favored, leading to a closer interaction and dependency where the plant through radial exudates supplies an energy source to the fungus, which increases exploration surface in the plant root system and improves nutrient absorption, especially of P (Osorio, 2011; Osorio *et al*., 2017). The P levels in soil solution and foliar tissue in the host are so important that they can activate or deactivate P transporters in mycorrhizal hyphae, directly affecting P plant absorption by this mechanism (Zandavalli *et al*. 2004; Osorio, 2011; Osorio *et al*., 2017).

Growth and development of *Gmelina arborea* inoculated with five strains of AMF in P levels in soil solution

The height (P<0.05) of *G. arborea* was found in treatments with inoculation of *R. fasciculatum*, followed by *R. aggregatum*, *R. irregulare*, *G. fistulosum*, and *E. colombiana*, for P levels in a soil solution of 0.02 and 0.002 mg L^{-1}, respectively. Meanwhile, for 0.2 mg L^{-1} of P in soil solution and control (no-inoculated plant), the values were not statistically different (P>0.05) and presented the lowest height (P<0.05) (Figure 3A).

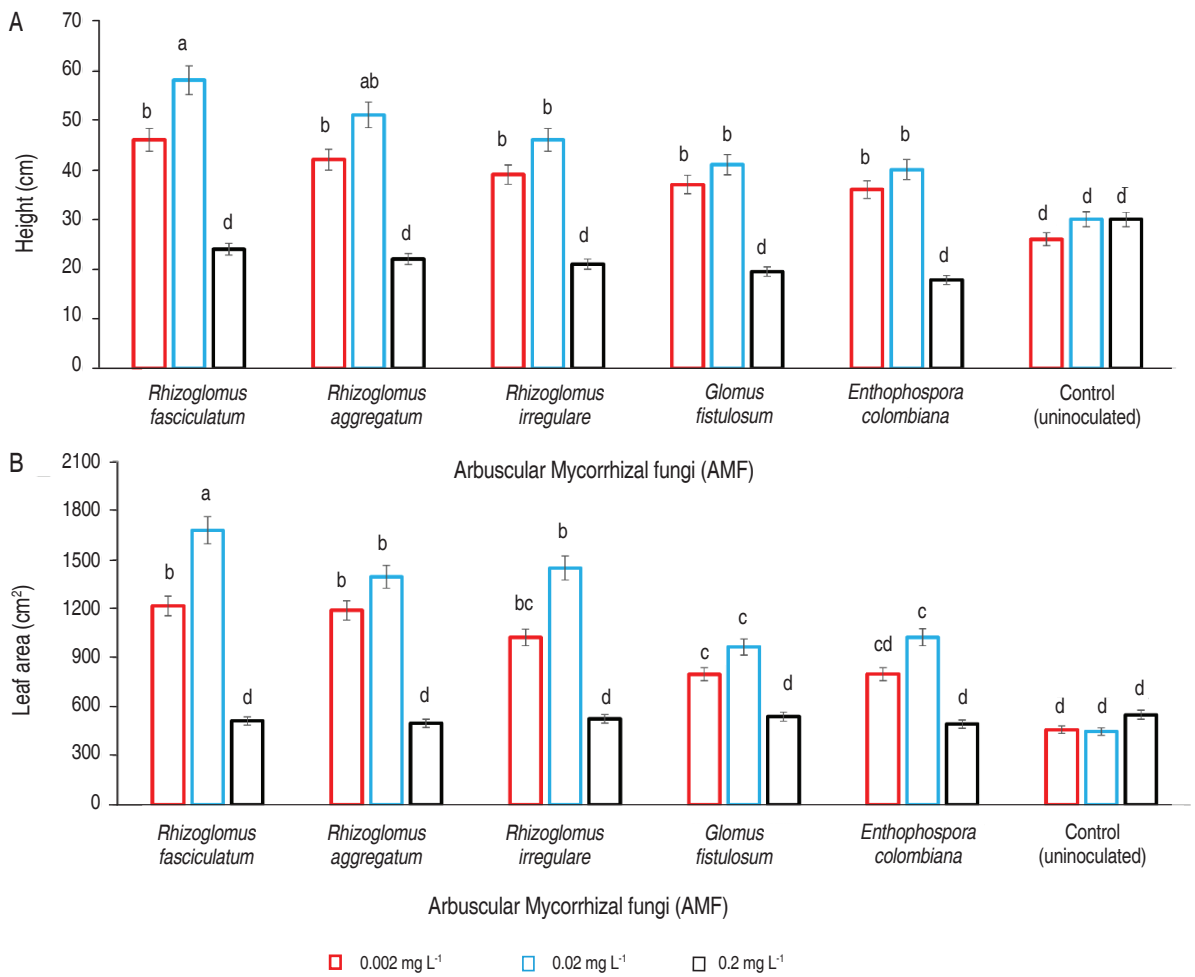

Figure 3. A. Height; B. leaf area of *Gmelina arborea* inoculated with five species of arbuscular mycorrhizal fungi. P levels evaluated in the soil solution were 0.002, 0.02 and 0.2 mg L^{-1}. Statistical analyzes were performed for each level of P and each AMF species evaluated. Equal letters on the bars represent no significant differences with a significance level of 95%. Lines on the bars represent standard error of the mean.

Leaf area presented same behavior that height, where the greatest values (P<0.05) were for P level of 0.02 mg L^{-1} in soil solution, and inoculated with *R. fasciculatum* (1668 cm^2), followed by *R. aggregatum* (1394.4 cm^2) and *R. irregulare* (1447.4 cm^2). No statistical differences were found (P>0.05) among *G. fistulosum* (964.2 cm^2) and *E. colombiana* (1024.4 cm^2), and the control (447.3 cm^2) presented the lowest values (P<0.05) (Figure 3B). Total biomass was significantly greater (P<0.05) when *G. arborea* was inoculated with AMF species in the soil that contained 0.02 mg L^{-1} of P, followed by P dose of 0.002 mg L^{-1} and with less value when P concentration was 0.2 mg L^{-1} (Figure 4A). Concerning the AMF strains evaluated, the biggest biomass (P<0.05) was obtained when plants were inoculated with *R. fasciculatum*, followed by inoculation of *R. aggregatum*, *R. irregulare*, and *G. fistulosum*. Meanwhile, treatment with *E. colombiana* did not show a statistical difference (P>0.05) with *G. fistulosum*, with these two strains the biomass was inferior (P<0.05) to the other three *Rhizoglomus* species, but superior (P<0.05) to the uninoculated plants (control) (Figure 4A).

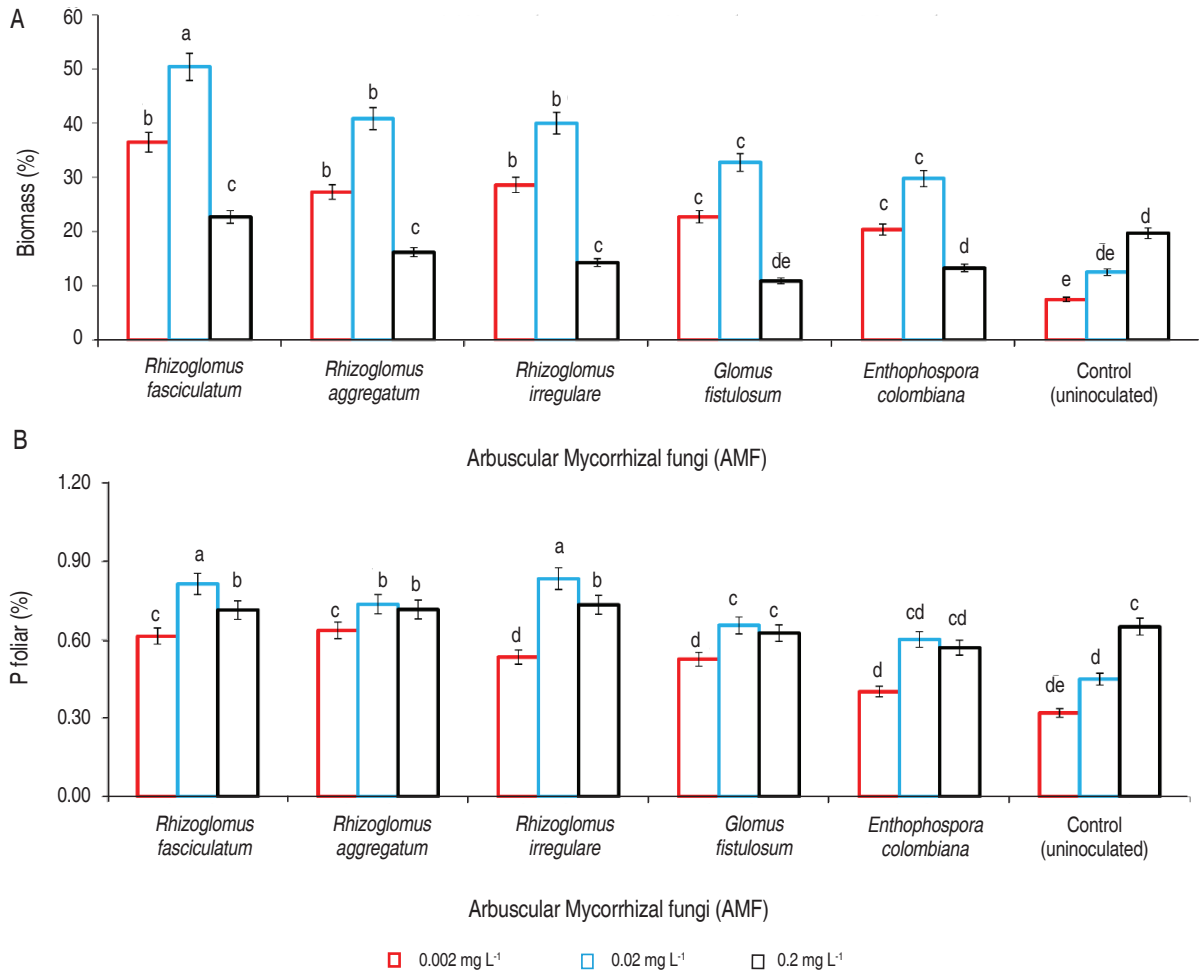

Figure 4. A. Biomass; B. foliar P of *Gmelina arborea* inoculated with five species of arbuscular mycorrhizal fungi. P levels evaluated in the soil solution were 0.002, 0.02 and 0.2 mg L^{-1}. Statistical analyzes were performed for each level of P and each AMF species evaluated. Equal letters on the bars represent no significant differences with a significance level of 95%. Lines on the bars represent standard error of the mean.

In the case of foliar P (Figure 4B) the highest concentration (P<0.05) was found when the soil had a P level of 0.02 mg L^{-1} and the plants were inoculated with *R. fasciculatum* and *R. irregulare*. They were followed by treatments associated with the same P concentration and the other inoculated AFM strains. Control and

plants inoculated with all strains under phosphorus level of 0.2 mg L^{-1} did not show statistical differences (P>0.05) to the group above mentioned. All treatments associated with AMF inoculation and a P soil level of 0.002 mg L^{-1} were classified as a third group. The lowest P amount (P<0.05) were presented in the control with a P concentration of 0.002 and 0.02 mg L^{-1} (Figure 4B).

Same to MC and MD, growth and development of *G. arborea* was associated with P concentration in soil solution and specific AMF species inoculated. The highest values were obtained under 0.02 mg L^{-1} of P in the soil solution and *R. fasciculatum* presence. On the other hand, plant growth and development improved with all AMF treatments, and a higher P concentration in plant leaves was found concerning the plants that were not inoculated. These results agree with Hernández and Salas (2008) findings, indicating that inoculation of *G. arborea* with *R. fasciculatum* presented an increase in height, diameter, foliage weight, and roots of 10, 6.2, 6.7 and 44.7%, respectively. It also showed an improvement in inoculated seedlings behavior at nursery stage when transplanted to field.

Many comparative advantages were found in *G. arborea* when it established a positive symbiotic relation with AMF. Given these interactions, the plant improves its ability to increase the P absorption among other beneficial effects not evaluated in this study, especially under low P levels in soil solution (Osorio, 2011, Osorio *et al*., 2017). This effect has been evaluated in many other species of economic importance for the Colombian tropics, in which all show better growth and development when the symbiotic relationship is successfully generated (Osorio, 2011; Osorio *et al*., 2017; Ramírez-Gil *et al*., 2013; Ramírez-Gil *et al*., 2014; Ramírez-Gil *et al*., 2015).

This work is a pioneer in reporting a detailed study of the interactions between *G. arborea* and five AMF species, it is a fundamental part of the search process for sustainable alternatives for fertilization, where the use of AMF may compensate the low nutrient levels in tropical soil and systems, especially P (Randhawa *et al*., 2006; Osorio, 2011; Osorio *et al*., 2017). The use of this biotechnology may help to decrease the use of chemical fertilizers with many economic and environmental benefits since the frequent use of these compounds increase production cost and could have adverse effects on the environment (Brady and Weil, 1999).

The inoculation of the strains *R. fasciculatum*, *R. aggregatum*, and *R. irregulare* is recommended during nursery stage which may increase the yield of the productive systems with this species since its cultivation is carried out in Colombia with low levels of natural fertility (Cadena and Guauque, 2009). Besides, it shows a high capacity of P retention, limiting phosphoric fertilization because the phosphate ion is rapidly precipitated and absorbed (Osorio, 2011; Osorio *et al*., 2017).

Moreover, understanding that the symbiotic relations with AMF show comparative advantages such as better adaptability to adverse conditions like drought, tolerance to disease attack among others (Yano and Takaki, 2005; Ramírez-Gil *et al*., 2014; Osorio *et al*., 2017). It could be a solution to face adverse effects of climate change, each time more frequent and intense, by alleviating the devastating effects on some vegetable species, especially those which are in tropical zones where climate variability is a problem that threatens the sustainability of different production systems.

CONCLUSIONS

The interaction of *G. arborea* and the five AMF species evaluated favored its development under low values of P (0.02 and 0.002 of mg L^{-1}). The best results were obtained with *R. fasciculatum*, *R. aggregatum* and *R. irregulare* strains. It is important to continue the research with the objective of improving AMF usage in forest plantation in order to improve the sustainability in tropical areas with infertile soils.

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Identification of resistance sources of common bean (*Phaseolus vulgaris* L.) to angular leaf spot (*Pseudocercospora griseola*)

Identificación de fuentes de resistencia del frijol común (*Phaseolus vulgaris* L.) a la mancha angular (*Pseudocercospora griseola*)

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Keywords:
Common bean germplasm
Genetic resistance
Inoculation
Plant breeding
Pyramid genes

ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is the most important edible legume in the world and is an important source of income for farmers and food for millions of families. Angular Leaf Spot (ALS), caused by the fungus *Pseudocercospora griseola* (Sacc.) Ferraris, is one of the most important diseases in the production of common bean with the potential to cause significant seed yield losses. An effective and environmentally friendly alternative to control this disease is the use of improved varieties that combine resistance genes of Andean and Mesoamerican origin. In this study, the response of 181 bean genotypes (coming from different breeding programs of several countries including the USA, Puerto Rico, Honduras, Ecuador, Colombia, Tanzania, Malawi, and Angola) to two angular spot isolates (races 61:11 and 63:51, prevalent in Isabela and Juana Díaz, Puerto Rico) were evaluated. Many of these genotypes contained resistant genes to different biotic and to abiotic stress. A total of 16 lines were identified to have resistance to both races. The resistant lines include the Andean breeding lines CAL 143, 277 and the Mesoamerican cultivar 'Ouro Negro' that possess the resistance genes *Phg-5*, *Phg-1*, *Phg-3*, respectively. This information can help common bean breeding programs to pyramid genes from the Andean and Mesoamerican gene pools to generate varieties with long-lasting resistance to this disease.

Palabras clave:
Germoplasma de frijol común
Resistencia genética
Inoculación
Fitomejoramiento
Piramidación de genes

RESUMEN

El frijol común (*Phaseolus vulgaris* L.) es la leguminosa comestible más importante en el mundo, ya que constituye una fuente importante de ingresos económicos para los agricultores y de alimento para millones de familias. La mancha angular (ALS, *por sus siglas en inglés*), causada por el hongo *Pseudocercospora griseola* (Sacc.) Ferraris, es una de las enfermedades más importantes en la producción de este cultivo ocasionando pérdidas significativas en el rendimiento. Una alternativa de control efectiva y amigable con el medio ambiente de esta enfermedad es el uso de variedades mejoradas que combinen genes de resistencia de origen Andino y Mesoamericano. En este estudio se evaluó la reacción de 181 genotipos de frijol (procedentes de diversos programas de fitomejoramiento de varios países como EEUU, Puerto Rico, Honduras, Ecuador, Colombia, Tanzania, Malawi y Angola) a dos aislamientos de *P. griseola* (razas 61:11 y 63:51, predominantes en Isabela y Juana Díaz, Puerto Rico). Varios de estos genotipos poseen genes de resistencia a factores de estrés biótico y abiótico. Un total de 16 líneas con resistencia a ambas razas fueron identificadas. Las fuentes de resistencia incluyen las líneas Andinas CAL 143 y AND 277 y el cultivar Mesoamericano 'Ouro Negro' que poseen respectivamente los genes de resistencia a la MA *Phg-5*, *Phg-1*, *Phg-3*. La información obtenida de este estudio puede ayudar a los programas de fitomejoramiento en la piramidación de genes de los acervos genéticos Andino y Mesoamericano para generar variedades con resistencia durable a esta enfermedad.

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The common bean (*P. vulgaris* L.) is the most important edible legume on the planet. It is an important source of food for at least 300 million people who live mostly in developing countries. Common beans are especially important sources of nutrition for women and children, and the crop also generates income for millions of smallholders (Velásquez and Giraldo, 2005, cited by Torres *et al*., 2013). For this reason, common bean is in the eighth position among the pulse crops cultivated in the world (Torres *et al*., 2013). According to statistical data from the Food and Agriculture Organization of the United Nations (FAO), dry bean production in the world during 2012 reached 23.1 million tons in a cultivated area of 29.2 million hectares.

Common Bean production is affected by some diseases that are widespread in production areas worldwide (Ddamulira *et al*., 2014). Angular Leaf Spot (ALS), caused by the hemibiotrophic fungus *Pseudocercospora griseola* (Sacc.) Crous and Braun, is one of the most devastating diseases, causing yield losses of up to 80% (Singh and Schwartz, 2010). Genetic resistance is an effective and environmentally friendly strategy for disease management. However, the diversity and high virulence of *P. griseola* and the emergence of new races of this pathogen are a challenge for the development of cultivars with a long-lasting resistance (Sartorato and Alzate-Marin, 2004; Abadío *et al*., 2012).

According to Hernández-López (2013), there were two centers of domestication of common bean: one primary (Mesoamerica) and one secondary (Andean). The isolates of *P. griseola* have also been divided into Andean and Mesoamerican groups that correspond to the two groups of the common bean origin (Pastor-Corrales and Jara, 1995; Pastor-Corrales *et al*., 1998). The co-evolution of *P. griseola* and common bean offers the possibility of combining resistance genes from both gene pools (Andean and Mesoamerican) to achieve a long-lasting resistance.

P. griseola virulence is assessed using a system proposed by the International Center for Tropical Agriculture (CIAT) that is based on the reaction of the ALS isolated pathogen with a standard differential set of six Mesoamerican and six Andean common bean cultivars of diverse origin (Pastor-Corrales and Jara, 1995). Screening the standard differential set of genotypes with endemic isolates of the ALS pathogen provides information about the pathogenic variability of *P. griseola*. These findings are useful to determine the distribution and frequency of pathotypes (races) and to select the best sources of resistance for different geographic regions.

According to Souza *et al*. (2016), six ALS resistance genes have been identified: *Phg-1*, *Phg-2*, *Phg-2²*, *Phg-3*, *Phg-4*, and *Phg-5*. The *Phg-1*, *Phg-4*, and *Phg-5* genes are from an Andean origin, and the *Phg-2* and *Phg-3* genes are from a Mesoamerican origin. Continuing identification and evaluation of additional genotypes with broad resistance should increase the genetic diversity of the sources of resistance to this disease. Therefore, the objective of this study was to identify the most resistant genotypes from a group of 181 bean genotypes of diverse geographic origins when inoculated with two highly virulent races of *P. griseola.*

MATERIALS AND METHODS

The experiment was carried out in two greenhouses of the University of Puerto Rico (UPR). Two isolates of *P. griseola* collected in Puerto Rico, coded as ALS 9029JD2 (collected in the Juana Díaz locality) and ALS 1146C (collected in the Isabela locality), were used for this study. Based on their reaction to Angular Leaf Spot differentials (Pastor-Corrales and Jara, 1995), these isolates were characterized as races 61:11 and 63:51, respectively (Estevez de Jensen *et al*., 2015). Three groups of common bean genotypes were inoculated with these *P. griseola* isolates. The first group was the BASE 120 (Bean Abiotic Stress Evaluation) trial consisting of 118 lines of common bean and two of tepary bean (*Phaseolus acutifolius* L.) lines (Table 1). This group is composed mainly of Mesoamerican genotypes and a few of Andean origin from breeding programs in Puerto Rico, Honduras, Colombia, and the United States. The second group of common bean genotypes included 34 lines and varieties of Andean and Mesoamerican origin from Honduras, Ecuador, and Puerto Rico that were previously selected for resistance to *P. griseola* (Table 1). The third group of common bean lines included 27 lines and varieties from the Andean Diversity Panel (ADP) (Table 1). The ADP consists of 396 bean accessions

and includes important improved lines and local varieties that originated mainly from Africa, the Caribbean, and North and South America (Cichy *et al*., 2015). The 27 genotypes of the ADP used in this study were selected because they presented resistance to ALS in field evaluations in Cedara, South Africa (Cichy *et al*., 2015).

Table 1. Groups of common bean genotypes evaluated.

Groups	Nº of genotypes	Origin	Gene Pool
BASE 120	120	UPR, USDA-ARS-TARS, USDA-ARS, Michigan State University, University of Colorado, CIAT and EAP Zamorano	Mesoamerican mainly and a few Andean
Inbred lines and varieties	34	INIAP, UPR, EAP Zamorano	Mesoamerican mainly and Andean
Andean Diversity Panel (ADP)	27	Tanzania, Burundi, Angola, Malawi, East África, Puerto Rico (UPR), Ecuador (INIAP), Colombia (CIAT)	Andean mainly and few Mesoamerican
Total	181		

CIAT: Centro Internacional de Agricultura Tropical; EAP: Escuela Agrícola Panamericana; INIAP: Instituto Nacional de Investigaciones Agropecuarias.

The methodology of sowing, inoculation, and evaluation was the same for the three groups of bean genotypes. Each genotype was planted in a 9 cm diameter plastic bag containing commercial substrate (Sunshine mix #1). Five seeds of each genotype were planted and thinned to three seedlings in a pot after germination. Each pot was considered as an experimental unit. The inoculum consisted of the two *P. griseola* races increased in V8 culture medium (200 ml V-8 juice, 3 g CaCO3, 18 g Bacto agar and 800 mL sterile distilled water) following the methodology of Castellanos *et al*. (2011). The isolates were grown in a V8 agar media and incubated at about 24 °C for 15 d. The inoculations were carried out 15 d after sowing using the first trifoliate leaf by spraying the inoculum on the underside of the leaf. The inoculum concentration was 1×10^4 conidia mL^{-1} adjusted using a hemocytometer (1/400 square mm, Hausser Scientific). The inoculated plants were exposed to a relative humidity of 90-100%, using a humidifier for 72 h after the inoculation. Afterward, they were placed under benches in the greenhouse and submitted to constant humidity (80-90%) every night until evaluation.

The evaluation of ALS severity was scored after 15 d of inoculation according to the 1-9 CIAT scale, where 1= plants have no symptoms; 3= plants with 2% of the leaf surface with lesions; 5= plants with 5% of the leaf area with lesions and sporulation; 7= plants with up to 10% of the leaf surface with lesions and sporulation associated with chlorosis and necrotic tissues; 9= 25% of the leaf area with lesions, frequently associated with early leaf fall and plant death (Schoonhoven and Pastor-Corrales, 1987).

The severity observed in the first three groups of genotypes were classified as follows: plants with mean values from 1 to 3 were considered resistant; plants with values from >3 to ≤6 were considered to have intermediate resistance; plants with values from >6 to 9 were considered susceptible (Schoonhoven and Pastor-Corrales, 1987). At the time of the evaluation, the plants that presented leaves with lesions without the development of sinemas were exposed to humidity >80% for 24 h with a humidifier, and leaves from plants that did not develop sinemas after that treatment were considered resistant (Sartorato, 2002). Temperature and humidity were recorded using an iButton® sensor (Maxim Integrated TM, USA) every 15 min.

The data analysis was performed using the statistical software Infostat (version 2008). The experimental

design was a randomized complete block (RCBD) with two replications. Analyses of Variance (ANOVA) was completed using the General Linear Model with a significant level of $P<0.05$. Means were compared using Fisher's least significant difference with a significant level of $P<0.05$.

RESULTS AND DISCUSSION

The temperature ranged from 20 to 36 °C with an average of 26±3 °C, and the relative humidity ranged (during three days of continuous humidification after inoculation) from 60-79% during the day, and 80-90% during the night. Allorent and Savary (2005) presented different limits in temperature ranges for each stage of the disease development. Spore germination, disease development, and sporulation can occur between 12 and 30 °C. In this research, the inoculations were conducted in the afternoon (after 5 PM) to favor the initiation of infection process because temperatures were <30 °C during this time. However, the temperature increased up to 36 °C during short periods, mainly at midday, which exceeded by 3 °C the maximum temperature indicated for the development of this disease. During the evaluation, susceptible checks showed symptoms with abundant sporulation; thus temperatures higher than 33 °C during short periods did not affect the development of the disease. The provision of high humidity (using humidifiers) during the evaluations was critical for the development of the disease.

There were significant differences between lines of reaction to both isolates in all inoculated trials (Table 2) because of the lines in the three groups of genotypes presented different levels of disease severity for each isolate. There were a small number of lines (<20) with resistance to both isolates.

In the first group of genotypes (BASE 120) evaluated with the isolate ALS 9029JD2 (race 61:11) 60 lines were susceptible (>6.0), four lines had resistant scores (≤ 3.0), and 42 lines showed intermediate resistance (>3 and ≤ 6). With the isolate ALS 1146C (race 63:51), 55 lines were rated as susceptible, four lines were resistant, and 48 lines had intermediate resistance. Only the lines G21212 and SER125 were resistant to both isolates.

Table 2. Summary of ANOVA for the severity of two isolates of angular leaf spot in three bean lines groups.

Groups of bean genotypes	Isolate code	*P*-value	Mean disease score[1]	CV (%)
BASE 120	ALS 9029JD2	<0.0001	6.32	12.25
Improved Lines/varieties		<0.0001	3.84	13.71
ADP		<0.0001	5.16	12.89
BASE 120	ALS 1146C	<0.0001	6.00	11.15
Improved Lines/varieties		<0.0001	4.30	21.82
ADP		<0.0001	5.88	12.63

[1] Rated according to the 1-9 CIAT scale (Schoonhoven and Pastor-Corrales, 1987); CV: Coefficient of variation.

In the second group of genotypes (the bean lines and varieties developed by various breeding programs for resistance to angular leaf spot), 15 lines were found to be resistant to the ALS 9029JD2 isolate, 11 lines had intermediate resistance, and eight lines were susceptible. With the isolate ALS 1146C, 15 lines were found to be resistant, 13 lines had intermediate resistance, and six lines were found to be susceptible. 12 lines with resistance to both angular leaf spot isolates were identified: Ouro Negro, INIAP 484 Centenario, INIAP 483 Intag, AND 277, PR 0637-6, PR 1530-57, ALS 0546-78, ALS 0532-6, ALS 0531-41, ALS 0531-97, ALS 0546-60, and ALS NIL 604-29.

In the evaluation of the lines from the Andean Diversity Panel (ADP) inoculated with the ALS 9029JD2 isolate, 10 lines were resistant, five lines showed intermediate resistance and 11 lines were identified as susceptible. Inoculations with the ALS 1146C isolate identified four resistant lines, seven lines with intermediate resistance

and 12 susceptible lines. Only two lines, CAL 143 and AFR 612, were resistant to the two angular leaf spot isolates.

In summary, 16 bean genotypes, from a total of 181 genotypes evaluated, had a mean score ≤3.0; which classified them to be resistant to both isolates of *P. griseola* (Table 3).

These results demonstrate the genetic vulnerability of most of the bean lines to this disease, which has been mentioned previously by several authors such as Singh and Schwartz (2010), Abadio (2012), E Silva (2008), and Mahuku (2003) among others.

It is important to note that from the 16 bean genotypes identified as resistant (originated from breeding programs in Honduras (EPZ), Puerto Rico (UPRM), Colombia (CIAT), and Ecuador (INIAP)), some of them have resistance to other diseases of economic importance. For example, CAL 143, INIAP 484 Centenario, INIAP 483 Intag, and Ouro Negro showed resistance to several races of *Colletotrichum lindemuthianum* (Rodríguez-Ortega *et al*., 2018; Zuiderveen *et al*., 2016).

Table 3. Common bean genotypes with resistance to Angular Leaf Spot isolates ALS 9029JD2 and ALS 1146C.

Panel	Genotype	Pool gene[a]	Color and size of seed	Origin
BASE 120	G21212	MA	Black, small	CIAT-Colombia
	SER 125	MA	Red, medium	CIAT-Colombia
Improved Lines and Cultivars	Ouro Negro	MA	Black, small	Brasil
	INIAP 484 Centenario+	A	Red mottled, large	INIAP-Ecuador
	INIAP 483 Intag+	A	Purple mottled-large	INIAP-Ecuador
	AND 227+	A	Red mottled, large	CIAT-Colombia
	PR 0637-6+	A	Red mottled, large	UPR
	PR 1530-57	MA	White, small	UPR
	ALS 0546-78	MA	Black, small	Zamorano
	ALS 0532-6	MA	Red, small	Zamorano
	ALS 0531-41	MA	Red, small	Zamorano
	ALS 0531-97	MA	Red, small	Zamorano
	ALS 0546-60	MA	Black, small	Zamorano
	ALS NIL 604-29	MA	Red, small	Zamorano
ADP	CAL 143+	A	Red mottled, large	CIAT
	AFR 612+	A	Red mottled, large	Malawi

[a]: A= Andean genotypes, MA= Mesoamerican genotypes

Because of the high variability of *P. griseola*, the improvement for effective and lasting resistance to angular leaf spot requires the introduction of resistance genes of Andean and Mesoamerican origin (Mahuku *et al*., 2003). Therefore, the different combinations of the resistance genes present in the 16 genotypes identified as resistant in this study should provide wider and long-lasting resistance. Although the groups of lines were evaluated with two highly virulent isolates of *P. griseola* (races 61:11 and 63:51), the resistant lines should be screened with other endemic isolates of the ALS pathogen. Information about the pathogenic variability of *P. griseola* is useful to determine the distribution and frequency of pathotypes (races) and to select the best sources of resistance for different geographic regions.

Genetic studies have reported two types of inheritance (qualitative and quantitative) of ALS resistance (Keller *et al*., 2015; Oblessuc *et al*., 2012). It is important to investigate the inheritance of the resistance present in these genotypes. This knowledge will help plant breeders optimize the selection of resistant plants. According to

Pereira *et al*. (2015), AND 277, Ouro Negro, and CAL 143 are recognized sources of resistance. Although these lines are not resistant to all isolates of *P. griseola*, they were resistant to the two races of *P. griseola* (61:11 and 63:51) from Puerto Rico. Souza *et al*. (2016) reported that these lines have the *Phg 1, Phg 3 and Phg 5* resistance genes respectively.

It is important to continue the evaluation and identification of new sources of resistance to expanding the genetic base of resistance to this disease in order to counter the pathogenic variability identified in *P. griseola*.

CAL 143 is considered a very important source of resistance to angular leaf spot that is widely used by plant breeding programs in several countries. For example, CAL 143 is a parent of the improved variety INIAP 484 Centenario (Table 9) in Ecuador (Murillo *et al*., 2012). This variety has shown resistance to thirteen angular leaf spot races identified in that country and its resistance has remained stable in the field until now (unpublished data).

Similar to CAL 143, other bean lines such as BAT332, G5686, MAR 2, MAR3, Mexico54, AND277, Cornell 49-242, and Ouro Negro, among others, are also considered important resistance sources (Souza *et al*., 2006; Gonçalves-Vidigal *et al*., 2013). Therefore, it is important to continue with the validation and identification of Quantitative Trait Locus (QTL) or resistance genes in these genotypes, and the molecular markers linked to them, which will facilitate their use by genetic improvement programs through marker-assisted selection.

CONCLUSIONS

Only 16 of the 183 genotypes evaluated were resistant to the two isolates *P. griseola* (races 61:11 and 63:51). The 16 resistant lines were: G21212, SER 125, AFR 612, Ouro Negro, CAL 143, AND 227, PR 0637-6, PR 1530 -57, ALS 0546-78, ALS 0532-6, ALS 0531-41, ALS 0531-97, ALS 0546-60, ALS NIL 604-29, INIAP 484 Centenario, INIAP 483 Intag. The last twelve lines were developed by breeding programs from CIAT (Colombia), the UPR (Puerto Rico), Zamorano (Honduras), and INIAP (Ecuador). The sources of resistance include genotypes of Andean and Mesoamerican origin. This information can help plant breeding programs to pyramid genes from both gene pools and to generate varieties with long-lasting resistance to this disease.

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Weed communities in the organic cultivation of fresh maize intercropped with legumes and coffee husk

Comunidad de malezas en el cultivo orgánico de maíz verde en asociación con leguminosas y cascarilla de café

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ABSTRACT

The objective of this study was to evaluate the phytosociology of the weed communities in maize intercropped with legumes and coffee husk in an organic production system, emphasizing on the relative importance of the weeds and their biomass. The experiment was implemented with the following treatments: T1 - Maize intercropped with *Phaseolus vulgaris* and weed mowing, T2 - Maize intercropped with *Crotalaria juncea* and weed mowing, T3 - Maize intercropped with *Canavalia ensiformis* and weed mowing, T4 - Maize intercropped with *Cajanus cajan* and weed mowing, T5 - Maize grown on soil covered with coffee husk (100 m^3 ha^{-1}) and manual weeding and T6 - Maize grown under conventional tillage system and manual weeding (control). The treatments were performed under a random block design with five replications each. A phytosociological analysis of the weeds was performed at stages V4, V8, and R1 to determine the relative importance (RI%) and biomass. The lowest biomass (11.6 g m^{-2}) of weeds occurred when maize was grown on soil covered with coffee husk. In total, 13 species of weeds were identified, being *Cyperus rotundus* L. the most prevailing species (%). From this study, it was determined that growing maize on soil covered with coffee husk is an alternative to suppress weeds in the organic fresh maize system when coffee husk is available.

Keywords:
Agroecology
Organic production systems
Phytosociology
Weed suppression

RESUMEN

El objetivo de este trabajo fue evaluar la fitosociología de las comunidades de malezas en un cultivo intercalado de maíz con leguminosas y cascarilla de café en un sistema de producción orgánica, haciendo enfasis en la importancia relativa de las malezas y su biomasa. El experimento constó de los siguientes tratamientos: T1 - de maíz intercalado con *Phaseolus vulgaris* y siega de malezas, T2 - maíz intercalado con *Crotalaria juncea* y siega de malezas, T3 - maíz intercalado con *Canavalia ensiformis* y siega de malezas, T4 - maíz intercalado con *Cajanus cajan* y siega de malezas, T5 - maíz cultivado en suelo cubierto con cáscara de café (100 m^3 ha^{-1}) y deshierbado manual y T6 - Maíz cultivado bajo un sistema de labranza convencional y deshierbado manual (control), dispuestos en un diseño de bloques al azar con cinco repeticiones. El análisis fitosociológico de las malezas se realizó en los estadios V4, V8 y R1 para determinar la importancia relativa (RI%) y la biomasa. La menor biomasa (11,6 g m^{-2}) de malezas ocurrió cuando el maíz creció en suelo cubierto con cascarilla de café. Se identificaron un total de 13 especies de malezas, siendo *Cyperus rotundus* L. la especie más predominante (%). A partir de este estudio, se determinó que cultivar maíz en suelo cubierto con cascarilla de café es una alternativa para suprimir las malas hierbas en un sistema orgánico de maíz fresco, cuando las cáscaras de café están disponibles.

Palabras clave:
Agroecología
Sistema de producción orgánico
Fitosociología
Supresión de malezas

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The management of weeds without the use of agrochemicals is one of the challenges of the organic production systems, which depends on the intensity of the interference, the floristic formation, period, and intensity of the infestation (Fialho *et al*., 2010). Usually, in this type of systems, the chemical method is replaced by mechanical and/or cultural methods, as well as other methods such as brushing, weeding, fertilization and irrigation, and duration of coexistence time (Fialho *et al*., 2011).

The modern agriculture systems rely on the economic system and require the use of external inputs based on mineral fertilizers and agrochemicals, being characterized by the simplification of agroecosystems (Gonçalves, 2014; Abreu *et al*., 2012). Opposite to this modern farming, the organic production system is based on the crop consortium. This practice is mainly used by family farmers because its adaptation to the characteristics of family-run properties, the heterogeneity of products grown in the same area, less dependence on external resources, less need of capital, greater hand absorption of family work, and an ecological adjustment (Sediyama *et al*., 2014).

In comparison to the monoculture practice, the consortium presents some advantages, such as the optimization of inputs and manpower. The consortium can provide better use of production resources and reduce pest and disease problems and can control weeds (Guedes *et al*., 2010; Araújo *et al*., 2012).

Coffee production in the state of Minas Gerais was estimated at 30,724,085 sacks in 2016, which placed Brazil as the largest producer and exporter in the world (Conab, 2016). In this region, coffee is one of the major crops. The large coffee production of Minas Gerais is being held in the region of Zona da Mata Mineira. One of the residues of this high production is the coffee husk which, due to is availability, is used as fertilizer in the crop production (Caldeira *et al*., 2013) and the weed suppression.

As the coffee husk availability is high, it can be used as fertilizer for other types of crops. Since there are no studies that evaluate the use of coffee husk applied to the soil surface on fresh maize production, the objective of this study was to evaluate the phytosociology of the communities of weeds in maize intercropped with legumes and coffee husk in an organic production system, placing emphasis on the relative importance of the weeds and their biomass.

MATERIALS AND METHODS

Experimentation area and treatments performed

The experiment was carried out at the Experimental Station of Coimbra-MG (20°45'00.0"S 45°51'00.0"W) of the Universidade Federal de Viçosa at an altitude of 650 m in the Zona da Mata (Mesoregion of Minas Gerais). The soil of the experimental area is classified as dystrophic Yellow Red Argissol, terrace phase, clay texture (EMBRAPA, 2011).

The soil chemical analysis (layer 0-10) showed the following results: pH in water 4.8, 35.8 mg dm^{-3} P, 161 mg dm^{-3} K, 2.8 $cmol_c$ dm^{-3} Ca, 1.1 $cmol_c$ dm^{-3} Mg, 0.00 $cmol_c$ $dm^{-3}$of Al^{3+}, 5.45 $cmol_c$ dm^{-3} H + Al, 4.31 $cmol_c$ dm^{-3} base sum (SB), 4.31 $cmol_c$ dm^{-3} of Effective CTC, 9.76 $cmol_c$ dm^{-3} of CTC Potential, 50% base saturation (V), 0% aluminum saturation index (m), 3.99 dag kg^{-1} of organic matter and 23.2 mg L of P-remainder. The determinations were made according to EMBRAPA (2011); pH (1:2.5 for soil: water), Ca, Mg and Al (extractor KCl 1N), P and K (extractor Mehlich 1) and extractable acidity (H + Al) extractant Calcium Acetate 0.5 mol L^{-1}.

The experiment was implemented with the following treatments:

T1: Maize intercropped with *Phaseolus vulgaris* (Common bean) and weed mowing.
T2: Maize intercropped with *Crotalaria juncea* (Sunn hemp) and weed mowing.
T3: Maize intercropped with *Canavalia ensiformis* (Jack bean) and weed mowing.
T4: Maize intercropped with *Cajanus cajan* (Dwarf pigeon pea) and weed mowing.
T5: Maize grown on soil covered with coffee husk and manual weeding.
T6: Maize grown under conventional tillage system and manual weeding (control).

The plot was constituted by six rows, totaling 24 square meters (5×4.8 m) with 12.8 m^2 of useful area (squareplot),

being evaluated the four maize central lines, discarding the border of 1 m.

Before sowing maize, it was performed a harrowing operation to minimize the population of weeds in the site of the experiment. Maize sowing was performed on February 24th, 2016 with a mechanized sowing machine. The open-pollinated maize variety Al Bandeirantes 1310 was sown at five seeds per meter with 0.80 m of spacing, aiming at the final population of 62,500 plant ha^{-1}. The sowing of the jack bean (*Canavalia ensiformis* (L.) DC) was carried out in the density of five plants per meter, simultaneously to maize planting, in the same line of planting, using manual seed sowing tool. For the sowing of common bean (*Phaseolus vulgaris* L.), five plants per meter were used in the same planting line, using the manual seed sowing tool. Sunn hemp (*Crotalaria juncea* L.) and dwarf pigeon pea (*Cajanus cajan* L.) were planted in the planting row of maize, requiring a thinning to 10 plants per meter, a quantity recommended by farmers.

In the T5 treatment, the coffee husk was distributed in the equivalent of 100 m^3 ha^{-1} on the soil surface of each plot, without incorporation before planting the maize.

An organic fertilization of maize was carried out when the crop was in the vegetative stage V4 (four fully expanded maize leaves), by applying 40 m^3 ha^{-1} of organic compost (made from bovine manure and maize husk, 44.84% moisture) next to the sowing row and it was not incorporated into the soil (Galvão *et al.*, 1999). The results of the chemical analysis of the compost based on the dry matter weight determined according to the methodology described by Kiehl (2010) were: 10.61 g kg^{-1} organic carbon, 1.10 g kg^{-1} total N, 9.6 C/N ratio, 0.38 g kg^{-1} P, 1.20 g kg^{-1} K, 0.94 g kg^{-1} Ca, 0.42 g kg^{-1} Mg, 0.53 g kg^{-1} S, 158 mg kg^{-1} Zn, 37686 mg kg^{-1} Fe, 239 mg kg^{-1} Mn, 68 mg kg^{-1} Cu, 13.1 mg kg^{-1} B, 018 g kg^{-1} Na, and pH 8.83 g kg^{-1}.

Weeds were cut twice using a brushcutter when maize had three and six leaves completely developed (V4 and V8) after phytosociological evaluations in the treatments intercropped with maize. Maize in monoculture with coffee husk applied in the surface of soil and maize in monoculture it was realized manual weed control at stages V2 (two fully developed maize leaves), V5 (five fully developed maize leaves) and V8 (eight completely developed maize leaves).

The average of the monthly temperature and rainfall was 20 °C and 48 mm, respectively, during the conduction of the experiment. Although there was rainfall during the experimental time, the cultures were supplemented via irrigation water.

Dry mass production of intercropped plants and weeds

After the maize harvest, the dry mass of jack bean (*Canavalia ensiformis* (L.) DC), common bean (*Phaseolus vulgaris* L.), dwarf pigeon pea (*Cajanus cajan* L.) and sunn hemp (*Crotalaria juncea* L.) were determined by sampling an area of 1 m^2. Samples of the plants were cut close to the soil; then they were placed in a drying oven with forced air ventilation at 70 °C for 72 h. After reaching constant weight, the samples were weighed, and the amount of dry mass was used to estimate the production per hectare of each treatment in the intercropped system. The same procedure was performed for weeds.

Phytosociological study of weeds plant communities

After planting the maize, samples of weeds were collected at three different stages: V4 (four fully developed maize leaves), V8 (eight fully developed maize leaves), and during the reproductive stage R1 (flowering). These evaluations were done before the mowing operation between the maize rows. Plants were collected using a 0.25 m side (0.0625 m^2), randomly placed between the lines of maize; then it was collected three samples per plot.

Samples of weeds were cut close to the soil, later identified according to species and family and then placed in a drying oven with forced air ventilation at 70 °C for 72 h to obtain the dry matter of the plant species evaluated. After registering the number and the dry matter value of the weeds, were determined the phytosociological parameters represented by the relative importance (RI%), following the methodology described by Pitelli (2000).

Data analysis

The descriptive analysis of the phytosociological parameters was represented by the relative importance (RI%). For the characteristics, dry mass from intercropped plants and weeds, the data were interpreted through the analysis of variance and the means compared by the Duncan test, at a significant level of 5%. The analyses were performed with the statistical program Assistat, version 7.7 (Silva and Azevedo, 2016).

RESULTS AND DISCUSSION

Dry mass production of intercropped plants and weeds

Among the intercropped plants, the jack bean resulted in the highest amount of dry matter, differing statistically from the other evaluated plants (Table 1).

The values found in the present study may be related to edaphoclimatic factors. Like the research done by Cesar *et al*. (2011) on the performance of green manure cultivated in two seasons of the year in the Cerrado of Mato Grosso do Sul, where values of dry mass yields varied according to the sowing time of the legume. Their study concluded that jack bean had higher dry mass values in the autumn-winter crop. However, in the present study, the sowing of the jack bean was carried out in a non-seasonal period.

The dry matter values of *C. juncea* L. in this study did not show relevance. According to Timossi *et al*. (2014), the best time for the cultivation of *Crotalaria juncea* L., targeting production of biomass, it is at the beginning of the rainy season.

Table 1. Dry mass production of intercropped plants. Coimbra - MG, Brazil, 2016.

Intercropped plants	Dry mass (Mg ha^{-1})
Common bean	0.402 b
Sunn hemp	0.996 b
Jack bean	2.188 a
Dwarf pigeon pea	0.352 b
Mean	0.985
CV (%)	49.35

The averages followed by the same letter do not differ statistically from each other. It was applied by the Duncan Test at the 5% significant level. CV: Coefficient of Variation.

The common bean presented low dry matter, which may be related to insects; especially, Cucurbit Bee (*Diabrotica speciosa*), whose attack caused significant loss of leaf area and probably contributed negatively to its development.

Table 2 shows data from the dry mass and the number of weeds in the phenological stages V4, V8, and R1. It was verified that for the evaluated characteristics there were significant effects for the dry mass of weeds in V8 and R1, and the number of weeds in V8 and R1.

Table 2. Dry mass of weeds and number of weeds in the V4 (vegetative stage of 4 leaves), V8 (8 leaves) and R1 (female flowering), as a function of cover crops, monoculture maize (control) and coffee husks. Coimbra-MG, 2016.

Treatments	Dry mass (g m^{-2})			Number of plants (plants m^{-2})		
	V4	V8	R1	V4	V8	R1
T1: Common bean	14.2^{ns}	54.8 ab	33.8 a	9.0^{ns}	16.2 a	10.7^{ns}
T2: Sunn hemp	15.1	66.8 a	36.5 a	9.4	13.5 ab	8.8
T3: Jack bean	13.4	45.3 bc	37.6 a	7.2	10.9 b	6.4
T4: Dwarf pigeon pea	14.7	35.9 cd	26.0 ab	8.5	9.5 bc	8.7
T5: Coffee husk	16.1	20.5 d	11.6 b	10.6	5.7 c	4.2
T6: Control	8.8	20.0 d	17.4 b	7.9	11.6 ab	4.9
Mean	13.7	40.5	27.2	8.8	11.2	7.2
CV (%)	57.9	31.2	40.6	47.4	32.2	54.6

The averages followed by the same letter do not differ statistically from each other. It was applied by the Duncan Test at the 5% of significant level. CV: Coefficient of Variation. ns: not significant

At the phenological stage V4, there was no statistical difference among the treatments for both dry mass and the number of weeds. At phenological stage V8, the dry mass of weeds in all treatments increased concerning the previous evaluation (V4), as well as the number of plants, which may be associated with regrowth of the weeds after weeding and mowing. The planting of intercropped maize with *Crotalaria juncea* L. differed from the treatments T5: Maize grown on soil covered with coffee husk, T6: maize grown under conventional tillage system, and T4: maize intercropped with *Cajanus cajan* L., resulting on a higher dry mass of weeds. The sowing time of the crop may have influenced these results, since the dry matter of *Crotalaria juncea* L. in this study was not efficient to suppress the weeds.

At the end of the evaluations, at the R1 stage, the T5 treatment differed from the other treatments, showing lower dry matter (11.6 g m^{-2}) of weeds. These results are related to the soil cover provided by the husk, which decreases the necessary incidence of light for the survival of the weeds. According to Gusmão *et al*. (2014), coffee husk has several applications, being mainly used as fertilizers and biocomposite. At V8 stage, maize grown on soil covered with coffee husk differed significantly from the other treatments, except for the maize intercropped with *Cajanus cajan* L. with weed mowing. This result can be interpreted as the effect of the cover on the weeds, which may have influenced in the inhibition of their germination.

The mowing performed in the intercropping treatments after the sampling of the weeds in the evaluated phenological stages, it was not efficient in the suppression of weeds. However, the efficiency of this brushing depends, for the most part, on weed species, on the repetition of cutting, and the stage of the plant's development (Queiroz *et al*., 2010). Planting maize in monoculture with coffee husk applied on the soil surface was highlighted by the potential to suppress weeds during the maize cycle in the organic system. So, it is presented as an effective management strategy for the suppression of weeds.

Phytosociological study of weed communities

It was identified 13 species of weeds in the three stages of maize (V4, V8, and R1), as follows: *Cyperus rotundus* L., *Bidens pilosa* L., *Emilia sonchifolia* (L.) DC., *Sonchus oleraceus* L., *Galinsoga parviflora* Cav., *Artemisia vulgaris* L., *Eleusine indica* (L.) Gaertner., *Digitaria horizontalis* Willd., *Euphorbia heterophylla* L., *Conium maculatum* L., *Ipomoea* sp., *Phyllanthus niruri* L., and *Oxalis latifolia* Kunth.

In the treatments, the RI% of nut grass (*Cyperus rotundus* L.) was higher in all stages (V4, V8, and R1). In the evaluated stages, all the treatments provided differences in the dynamics of the weeds and the phytosociological relationship.

Cyperus rotundus L. is a perennial species, with wide adaptability to many agricultural environments and with sexual and asexual reproduction capacity (Panozzo *et al.,* 2009). It is one of the most important weed species in the world due to its rapid reproduction and dissemination, yielding difficulties for its control (Araújo Jr *et al*., 2015).

After sampling weeds in the evaluated phenological stages (V4, V8, and R1), it was performed mowing in the intercropped treatments. The treatments of maize in monoculture with coffee husk applied on the soil surface and the treatment of maize in monoculture were performed under manual weeding. The stage of competition between weeds and culture can be modified according to the period in which the community is demanding a given resource (Agostinetto *et al*., 2008).

The application of phytosociological indexes is significant to infer the impact of management systems and agricultural practices on the growth and occupation activity of weed communities in agroecosystems. These indexes provided the knowledge of the most important weeds within the weed community, for which management alternatives or even modifications must be established in the system to enable its control (Marques *et al*., 2011). In the first evaluation, performed at V4 stage, the species with highest values of RI% were the *Cyperus rotundus L*., *Bidens pilosa* L., *Oxalis latifolia* Kunth, and *Artemisia vulgaris* L., showing variation of the RI% values according to the treatment applied.

The RI% of the *Bidens pilosa* L. and *Artemisia vulgaris* L. together (58.63%) presented a value similar to the one represented by *Cyperus rotundus* L. in the treatment of maize in monoculture with coffee husk. T5 treatment, Maize in monoculture with coffee husk applied on soil

surface, stood out, among the other treatments, in the capacity to suppress *Cyperus rotundus* L. in the first phytosociological evaluation (Figure 1). The suppression of the other weeds that appeared during the V4 stage was more efficient in the treatment of maize intercropped with common bean (T1).

Besides, to become more popular for representing low cost (Oliveira *et al*., 2012), the coffee husk has been studied for its allelopathic effect on weeds (Minassa *et al*., 2017). Although the *Canavalia ensiformis* (L.) DC had covered the soil, due to its higher dry matter value in comparison to the other treatments, possibly due to the low allelopathic effect on *Cyperus rotundus* L. and the other plants found in the experiment area, it did not allow a significant reduction of phytomass of this weed plant. In the evaluation carried out, in the V8 phenological stage, there was a similar proportion of plant numbers in the treatments compared to the first vegetative stage (V4), but with less representative RI.

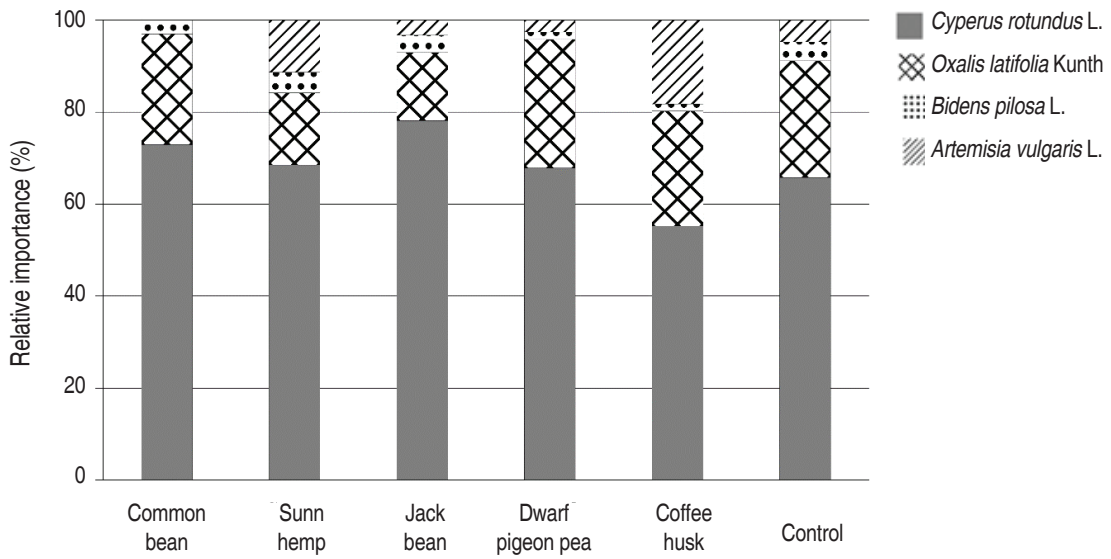

Figure 1. Graphical representation of the values of the relative importance of the plants in the growth V4 (vegetative stage of 4 leaves) maize. Coimbra - MG, 2016.

In the second phytosociological evaluation, the weeds that appeared with the highest frequency were: *Cyperus rotundus* L., *Bidens pilosa* L., *Oxalis latifolia* Kunth, and *Artemisia vulgaris* L. At this stage (V8), *Cyperus rotundus* L. remained the most important species among the treatments; however, there was a reduction in its RI%. The maize and common bean consortium (T1) was the least significant treatment of *Cyperus rotundus* L. (Figure 2). The data do not relate to the value of dry matter provided by the crop, probably due to the attack of *Diabrotica speciosa*.

At V8 phenological stage, it was observed the regrowth of the *Bidens pilosa* L. However, in the coffee husk treatment, there was a reduction of the *Bidens pilosa* L. with 6.75%. This reduction may have occurred due to the large area of soil covered with the coffee husk, which may have influenced the inhibition of germination of *Bidens pilosa* L., by smothering.

Maize grown on soil covered with coffee husk caused a greater suppressive effect on the other weeds in V8 phenological stage, but it was not efficient in the suppression of *Cyperus rotundus* L., compared to the first evaluation (V4). This result may be related to the decomposition of the coffee straw. The other spontaneous plants were affected by the maize shading. The most prevalent species in the reproductive stage were: *Cyperus rotundus* L., *Bidens pilosa* L., and *Oxalis latifolia* Kunth.

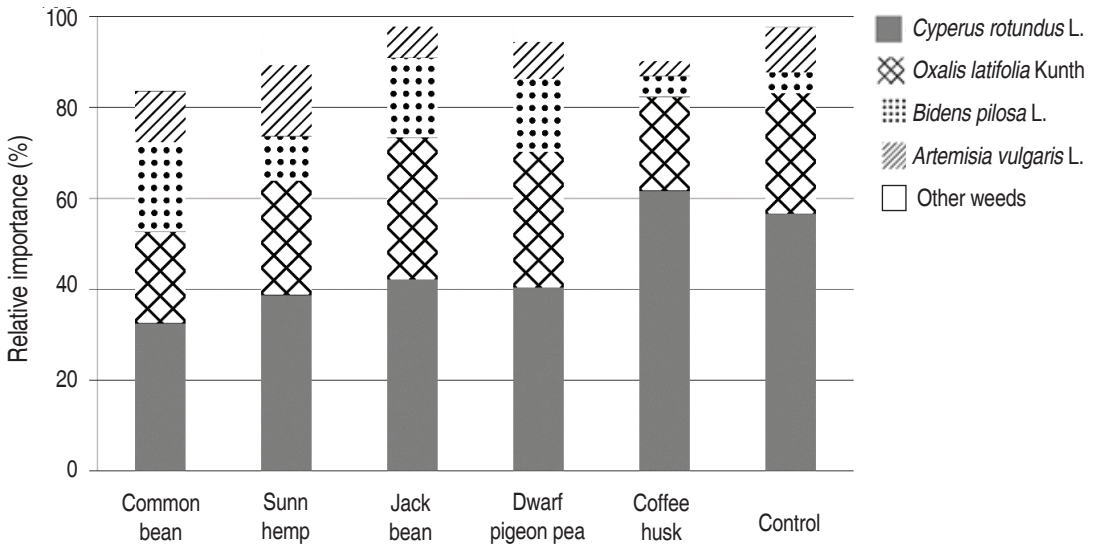

Figure 2. Graphical representation of the relative importance of weeds plants in the phenological stage V8 (vegetative stage of 8 leaves) of maize. Coimbra - MG, 2016.

At the end of the evaluations (V4, V8, and R1), *Cyperus rotundus* L. presented the highest RI%, being smaller in the consortium with dwarf pigeon pea. Regarding monoculture maize with coffee husk applied on the soil surface, it presented higher RI%; however, the treatment was efficient in the suppression of the other weeds (Figure 3).

In the third evaluation, at the R1 stage (flowering), the *Canavalia ensiformis* (L.) DC was not efficient in the suppression of weeds. These circumstances caused the decline of the suppression, which increased the emergence and growth of the weeds. The same fact may have occurred in the present study.

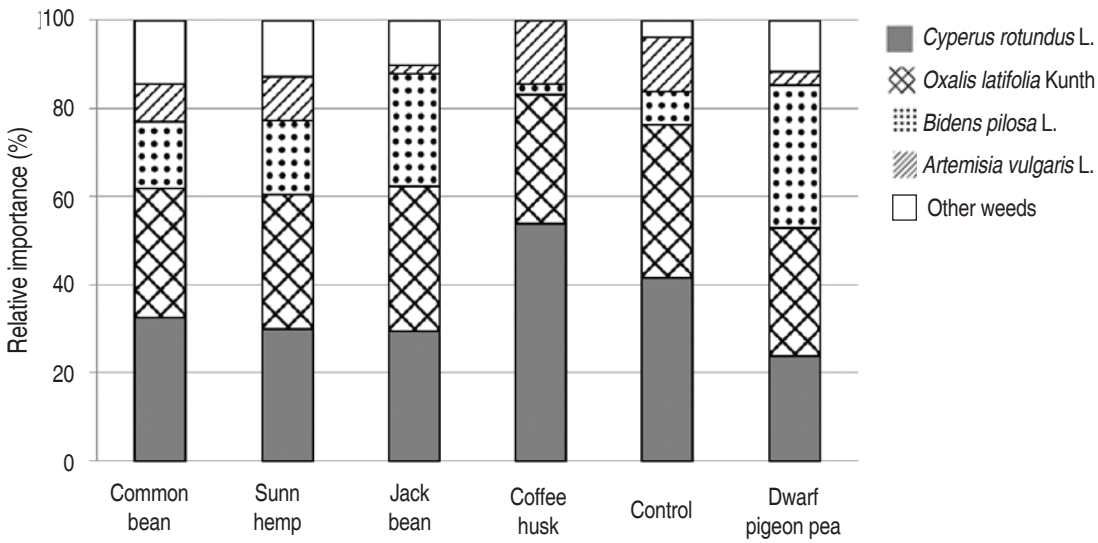

Figure 3. Graphic representation of the values of the relative importance of weeds plants populations in maize growth R1(female flowering). Coimbra - MG, 2016.

Cyperus rotundus L., *Bidens pilosa* L., *Oxalis latifolia* Kunth and *Artemisia vulgaris* L. were the species present in the three phenological stages (V4, V8, and R1). The monitoring of the population of weeds in intercropped systems can evaluate the allelopathic capacity between both plants, which makes possible to improve the production system, both adequately and economically, by reducing herbicide applications.

CONCLUSIONS

Growing maize in monoculture with coffee husk applied on the soil surface is an alternative to suppress weeds on organic fresh maize system. The knowledge about alternative methodologies concerning the chemical use, which efficiently manage the weeds, can help the decision of better planning to execute agricultural operations that benefit soil conservation. Among the evaluated weeds, *Cyperus rotundus* L. was the weed of highest relative importance (RI%) in organic maize culture.

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Influence of temperature on the occurrence of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) parasitoids in tobacco crops in Rio Grande do Sul, Brazil

Influencia de la temperatura en la ocurrencia de parasitoides de *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) en cultivos de tabaco en Rio Grande do Sul, Brasil

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ABSTRACT

The aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) is considered one of the main pests in tobacco crops. By knowing their natural enemies, such as parasitoid wasp, is the first step to develop management strategies for the biological control of the aphids using local agents. For the success of using this tool, it must be considered some environmental factors like thermal tolerance. Therefore, the objective of this work was to survey the occurrence of the parasitoids of *M. persicae* associated with tobacco crops in the state of Rio Grande do Sul, Brazil, as well as to evaluate the influence of temperature on the occurrence of these parasitoid species. During four crop seasons, tobacco leaves infested with aphids were collected in 42 cities of Rio Grande do Sul. The leaves with aphids were conditioned in plastic containers for ten days for later screening and verification of parasitoids' emergence. In total, 2963 individuals of two emerging species were sampled: 78% were *Aphidius colemani* Viereck (Hymenoptera: Braconidae), and 22% were *Praon volucre* (Haliday) (Hymenoptera: Braconidae). Among the 42 cities sampled, the occurrence of parasitoids was detected in 25 of them. Under the conditions of this study, it was confirmed the influence of the temperature on the populations of the parasitoids of *M. persicae.* Individuals of *P. volucre* occurred preferably in temperatures below 22 °C, unlike to *A. colemani*, which preferred higher temperatures, above 22 °C, showing a different thermal tolerance between both species.

Keywords:
Aphids
Aphidius colemani
Nicotiana tabacum
Parasitoid
Praon volucre
Temperature

RESUMEN

El pulgón *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) es considerado una de las principales plagas en cultivos de tabaco. Conocer sus enemigos naturales, como las avispas parasitoides, es el primer paso para desarrollar estrategias de manejo para el control biológico de los pulgones utilizando agentes locales. Para el éxito en la utilización de esta herramienta, se debe tener en cuenta algunos factores ambientales como la tolerancia térmica. En ese sentido, este trabajo tuvo como objetivo realizar una evaluación de la ocurrencia de los parasitoides de *M. persicae* asociados al cultivo del tabaco en el estado de Rio Grande do Sul, Brasil, así como, evaluar la influencia de la temperatura en la ocurrencia de estas especies parasitoides. Durante cuatro temporadas de cultivo, se realizaron colectas de hojas de tabaco infestadas con pulgones en 42 ciudades de Rio Grande do Sul. Las hojas con pulgones fueron acondicionadas en recipientes plásticos por diez días, para posterior identificación y verificación de la emergencia de los parasitoides. Se muestrearon un total de 2963 individuos emergidos, de dos especies: 78% a *Aphidius colemani* Viereck (Hymenoptera: Braconidae) y 22% a *Praon volucre* (Holiday) (Hymenoptera: Braconidae). De las 42 ciudades muestreadas, se detectó la presencia de parasitoides en 25 de ellas. En las condiciones en que se realizó el estudio, se constató que la temperatura ejerció influencia directa sobre las poblaciones de parasitoides de *M. persicae.* Los individuos de *P. volucre* ocurrieron preferentemente en temperaturas inferiores a los 22 °C, a diferencia de *A. colemani*, que presentaron preferencia por temperaturas mayores a los 22 °C, observándose una tolerancia térmica diferente entre las dos especies.

Palabras clave:
Áfidos
Aphidius colemani
Nicotiana tabacum
Parasitoide
Praon volucre
Temperatura

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Tobacco (*Nicotiana tabacum* L. Solanaceae) is cultivated for commercial purposes worldwide. In Brazil, its production is concentrated in the South region, and the state of Rio Grande do Sul has the largest planted area (dos Santos *et al*., 2017). This crop has great economic importance in the region due to the high commercial value and the capacity to employ a large number of people in both cultivation and industrialization (de Carvalho *et al*., 2014).

Brazil is now the second largest producer of tobacco leaves, after China, and has maintained a global leadership in export for two decades (Kist, 2014). On average, 85% of the Brazilian crop is shipped to more than a hundred countries in all continents (dos Santos *et al*., 2017).

During the vegetative development of tobacco's field, its leaves can be attacked by a range of pest insects, among them aphids of the *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) species, which are one of the most important pests due to a negative interference with the production and quality of tobacco (Kanavaki *et al*., 2006; Burrack, 2015). This insect presents a high reproductive capacity and dispersion. It can settle in the crop in a short time, causing serious damage by the continuous sucking of the sap and transmission of diseases (Backer *et al*., 2015).

There is a lack of information regarding the level of damage caused by *M. persicae* to tobacco crops. The only data available are for the state of North Carolina, USA, which vary according to the stage of the crop development and whether, or not, viral diseases are considered endemic (Davis and Nielsen, 1999). According to the same source, the level of damage is characterized when 10% of the plants present at least 50 aphids in a leaf from the apical half to the pruning and 20% after, being lower in regions where the virus transmission by *M persicae* is recognized.

Currently, the control strategies for *M. persicae* depend on chemical products in Brazil. However, the indiscriminate use of such substances has diminished their efficiency mainly due to the emergence of a resistant population (Carvalho and Barcellos, 2012). One of the alternatives for the management of aphids is the implementation of biological control using natural enemies, such as parasitoid wasps, chiefly representatives of the Braconidae family, which are important agents of aphids' natural mortality in agricultural and natural environments (Cruz, 2007).

The knowledge about the occurrence of these agents of biotic mortality as well as their distribution in areas of the Neotropical region is fundamental (da Silva and de Brito, 2015). Such knowledge establishes the necessary bases for their importance to biological control studies using these organisms as a pest management tool (González and Burgos, 1997).

Environmental factors such as temperature may act positively or negatively on biological aspects of parasitoids (de Conti *et al*., 2010). According to this environmental factor, the success of biological control is directly related to the tolerance of natural enemies to temperature. It is possible that, for the control of a particular pest species, several species of parasitoids or individuals of the same species are needed; however, they can be adapted to different climatic conditions (Messenger and van den Bosch, 1971). Adaptability to climatic conditions is among the key factors influencing the success of parasitoids in biological control programs (Nascimento, 2011).

Thus, the objective of this work was to survey the occurrence of the parasitoids of *M. persicae* associated with the tobacco crop in Rio Grande do Sul, Brazil, as well as to evaluate the influence of temperature on the occurrence of parasitoid species.

MATERIALS AND METHODS

The survey of the parasitoids of *M. persicae* in Virginia tobacco was carried out during 2010, 2011, 2012, and 2013, where seasons lasted from October to December of each year. The main tobacco producing regions in Rio Grande do Sul were visited, totaling 42 cities (Table 1). The visited crops were managed conventionally using synthetic products such as fertilizers, herbicides, fungicides and insecticides throughout the growing process.

The methodology outlined by Kavallieratos *et al*. (2005) was adapted to this study. There was not set an experimental design, and tobacco leaves attacked by *M. persicae*, with different levels of infestation, were collected randomly.

Table 1. Cities of Rio Grande do Sul where tobacco leaf collections with infestations of *M. persicae* were carried out in each season to verify the occurrence of its parasitoids.

Crop	Cities
2010	Agudo; Cerro Branco; Paraiso do Sul; Vera Cruz
2011	Agudo; Anta Gorda; Arroio do Tigre; Arvorezinha; Candelária; Casca; David Canabarro; Dr. Ricardo; Estrela Velha; Gramado Xavier; Muçum; Paraíso do Sul; Relvado; Segredo; Sinimbu; Sobradinho; Venâncio Aires; Vera Cruz; Vespasiano Corrêa
2012	Amaral Ferrador; Barão do Triunfo; Camaquã; Canguçu; Cerro Grande do Sul; Chuvisca; Dom Feliciano; Forquetinha; Herval; Herveiras; Novo Cabrais; Passo do Sobrado; Pelotas; Piratini; Santa Cruz do Sul; Santa Tereza; São Jeronimo; São Lourenço do Sul; Sério; Sertão Santana; Sinimbu; Vale do Sol; Venâncio Aires; Vera Cruz
2013	Arroio do Tigre; Herveiras; Sinimbu; Vale do Sol; Venâncio Aires; Vera Cruz

The leaves were then stored in plastic bags and sent to the Laboratory of Entomology of the University of Santa Cruz do Sul (UNISC), where they were cut into squares (3×3 cm), without accounting for the density of aphids in them. The material was conditioned in plastic containers (9.5 cm long × 7 cm wide × 5 cm deep), acclimatized at 26±2 °C for 10 d for further screening and verification of parasitoids emergence.

The emerged parasitoids were identified at a species level according to Wharton *et al*. (1997) and Kavallieratos *et al*. (2001). Dr. Marcus Vinicius Sampaio, professor of the Federal University of Uberlândia, confirmed the identification of the specimens. Subsequently, the material was collected and stored in alcohol (70%) at the Entomological Collection of Santa Cruz do Sul (SESC).

In addition to the survey of the parasitoid occurrence, a correlation was made between the parasitoid species found and the temperature (°C) of the cities. For this purpose, the average temperature of spring was considered according to data obtained from Climate-Data.Org (2017). This temperature was used because it is the period of planting, flowering, and the emergence of tobacco in the South region of Brazil, and consequently it represents a higher incidence of aphids.

For the correlation analysis between the occurrence of parasitoids and the temperature, the data of the cities only were used when ten or more parasitoids emerged from the collected aphids during all the crop seasons. As the sample number was different in each city and season, the total proportion of individuals in each site was considered.

The distribution map of the cities visited was plotted using the CorelDRAW® X7. The regression models were constructed using SigmaPlot 11.0 software (SigmaPlot, 2008).

RESULTS AND DISCUSSION

During the four crop seasons studied, 2963 parasitoids emerged from *M. persicae*, of which 2305 (78%) were *Aphidius colemani* Viereck (Hymenoptera: Braconidae) and 659 (22%) corresponded to *Praon volucre* (Haliday) (Hymenoptera: Braconidae). Among the 42 cities visited, there was the occurrence of parasitoids in 25 of them (Figure 1). Summing up the data of the four seasons, *P. volucre* was the most abundant species in 13 cities and *A. colemani* in 12 (Table 2). The low occurrence of parasitoids or their absence in some cities can be justified by the small sample in some of them, either by not locating crops infested with aphids or due to the excessive use of chemical agents on the crops.

Silva *et al.* (2012) had already reported the occurrence of these parasitoid species in tobacco in Rio Grande do Sul. According to a bibliographical survey, *A. colemani* came from the Mediterranean and Central Asian regions. Since 1992, it has been marketed in several countries for the control of aphids in protected crops (van Lenteren, 1997). In the past, it was successfully used in southern Brazil

to control wheat aphids (Gassen and Tambasco, 1983), adapting to the climatic conditions. *Aphidius colemani* is considered a dominant species among those found in aphids in South America and presents a high potential as a biological control agent (Sampaio *et al*., 2007), corroborating the results of this study.

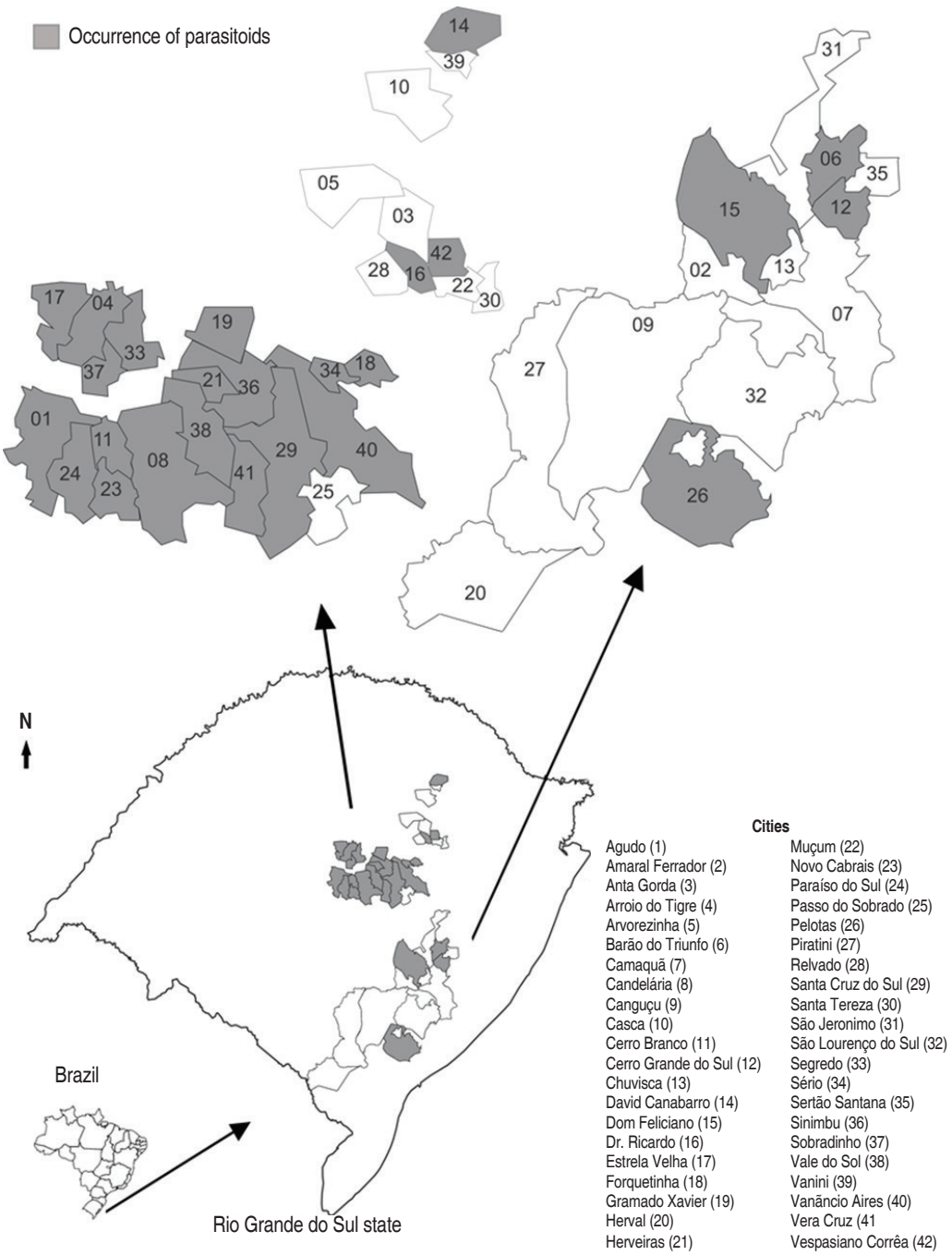

Figure 1. Cities in Rio Grande do Sul state where the surveys were carried out, highlighting the sites where there were occurrences of the parasitoids of *M. persicae* in tobacco crops.

Table 2. Number of parasitoids sampled in the cities of Rio Grande do Sul during four tobacco crop seasons.

Temp. (°C)	Cities	Crop				Total	
		2010	2011	2012	2013	*A. colemani*	*P. volucre*
18.5	David Canabarro	-	12	-	-	4	8
18.8	Sério	-	-	5	-	0	5
18.9	Vespasiano Corrêa	-	3	-	-	3	0
19.1	Dr. Ricardo	-	1	-	-	1	0
19.4	São Lourenço do Sul	-	-	10	-	0	10
19.5	Herveiras	-	-	184	137	120	201
19.7	Dom Feliciano	-	-	2	-	2	0
20.0	Gramado Xavier	-	5	-	-	5	0
20.4	Estrela Velha	-	116	-	-	0	116
20.5	Barão do Triunfo	-	-	3	-	1	2
20.6	Segredo	-	1	-	-	0	1
20.6	Sobradinho	-	17	-	-	0	17
20.7	Arroio do Tigre	-	10	-	4	2	12
20.7	Cerro Grande do Sul	-	-	5	-	0	5
21.9	Sinimbu	-	0	171	16	26	161
21.9	Vale do Sol	-	-	24	26	13	37
22.0	Agudo	2	0	-	-	0	2
22.0	Cerro Branco	25	-	-	-	9	16
22.0	Forquetinha	-	-	3	-	0	3
22.1	Santa Cruz do Sul	-	-	478	-	478	0
22.2	Candelária	-	84	-	-	82	2
22.2	Novo Cabrais	-	-	34	-	34	0
22.2	Paraíso do Sul	94	0	-	-	93	1
22.2	Venâncio Aires	-	0	525	9	522	12
22.2	Vera Cruz	0	0	838	119	907	50

- No collected tobacco leaves attacked by *M. persicae* in this year.

On the other hand, endoparasitoid *P. volucre*, of Palearctic origin, was also introduced in Brazil for the control of wheat aphids, establishing itself and becoming part of the group of parasitoids with potential use as control agents of different aphid species in different crops (de Conti *et al*., 2008). Nowadays, *Praon volucre* is a cosmopolitan species of great importance for several crops, both in field conditions and in protected environments in Brazil (Silva *et al*., 2008). It may be related to the adaptation of the species to the different climatic conditions of each region.

In Greece, Kavallieratos *et al.* (2005) support that *A. colemani* and *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Braconidae) are the principal parasitoid species of *M. persicae* in tobacco. In contrast, Kavallieratos *et al.* (2004) found that *P. volucre* was the dominant parasitoid species of *M. persicae* in a different tobacco growing area of Greece, whereas *D. rapae* was not recorded in that area. According to Starý (1970), interspecific relations are influenced by the geographical distribution of parasitoids which also affects their occurrence.

With respect to the proportion of individuals in each municipality and the average temperature in spring, a correlation for temperature with respect to the proportion of *P. volucre* was verified (r^2=0.92), that is, with the

increase in temperature, there was a proportional decrease in the number of individuals of this species (Figure 2). For the occurrence of *A. colemani*, there was a correlation in which the proportional incidence of individuals of this species increased at higher temperatures (r^2=0.84)(Figure 3).

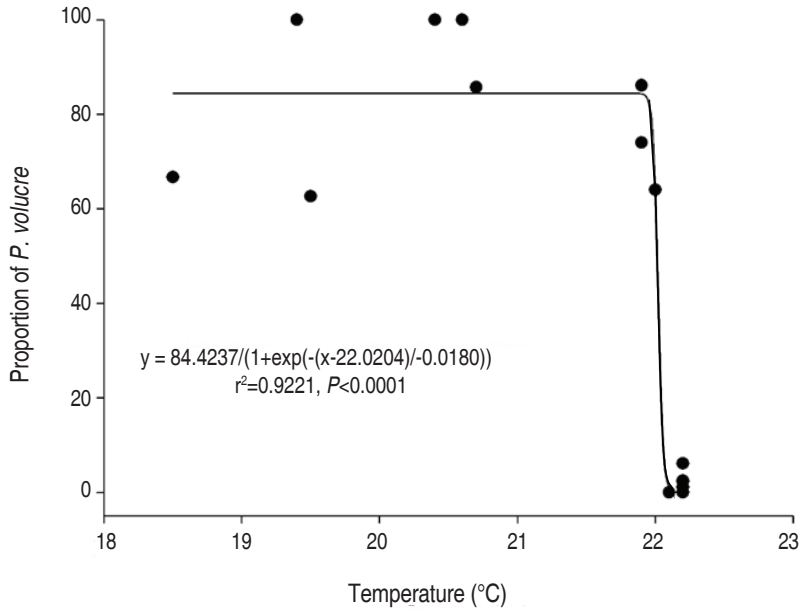

Figure 2. Correlation between the proportion of *P. volucre* emerged from *M. persicae* according to the different spring average temperatures of each city.

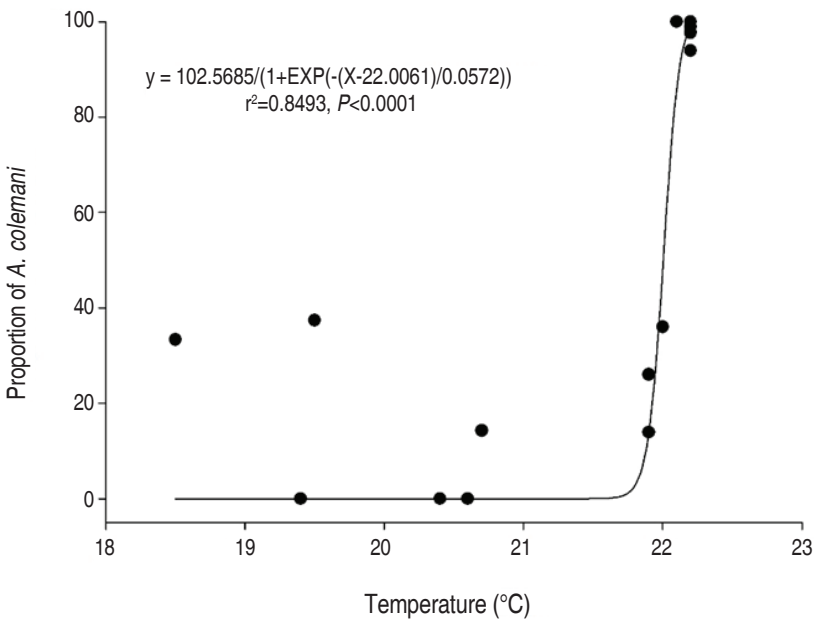

Figure 3. Correlation between the proportion of *A. colemani* emerged from *M. persicae* according to the different spring average temperatures of each city.

The results demonstrate that *P. volucre* presents a greater predominance in cities with average temperatures in spring equal to or lower than 22 °C (Figure 2). These results were also observed in the evaluation of the potential of *P. volucre* as an agent for the control of the aphids *Uroleucon ambrosiae* (Thomas) and *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) (de Conti *et al*., 2008; de Conti *et al*., 2010). High parasitism rates were observed at temperatures between 18 °C and 22 °C considering these climatic conditions favored mummification, emergence of parasitoids, and increasing in the longevity.

The parasitoid *A. colemani* presented predominance in cities with temperatures above 22 °C (Figure 3). This had also been observed by Zanini *et al*. (2006) in their study on aphids of the species *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae), and by Sampaio *et al*. (2007) and Sampaio *et al*. (2005) in their work on the development of *A. colemani* at different temperatures and different climatic regions. The species presented a high emergence of individuals at temperatures above 22 °C, being possible to report emergence at even higher temperatures in some warmer regions.

The fact that *A. colemani* has a higher tolerance at high temperatures may explain its predominance in agricultural environments in the southern region of Brazil. Based on the literature, *A. colemani* is formed by a species group, which are important biological control agents: *A. colemani*, *Aphidius transcaspicus* Telenga, and *Aphidius platensis* Brethes. This diversity can have an impact on the plasticity of the species in different environmental conditions (Tomanovic´ *et al*., 2014). This type of study is important to know the thermal limits of each species of parasitoids in order to infer the species most adapted to each climatic situation that in the future could be more effective as a tool in integrated pest management.

CONCLUSIONS

Two species of parasitoids *A. colemani* and *P. volucre* were surveyed on tobacco farms in Rio Grande do Sul, Brazil parasitizing *M. persicae*, being possible to infer that there is a variation in the occurrence of these natural enemies according to temperature.

Under the conditions of this study, temperature exerted a direct influence on the populations of parasitoids of *M. persicae.* Individuals of *P. volucre* occurred preferably at temperatures below 22 °C, unlike to *A. colemani*, which had a clear preference for higher temperatures, above 22 °C, showing a different thermal tolerance between both parasitoid species.

Therefore, the results demonstrate that there is a possibility of using the natural enemies found for the control of *M. persicae* in tobacco growing in Rio Grande do Sul state.

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Seed treatment with silicon on initial growth of soybean (*Glycine max*) cultivars

UNIVERSIDAD NACIONAL DE COLOMBIA

Tratamiento de semillas con silicio sobre el crecimiento inicial de cultivares de soja (*Glycine max*)

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ABSTRACT

Soybean (*Glycine max*) is a crop of high economic power in the world, being used to produce vegetable oil, as well as a source of food for animals and humans. Recent research indicates that nutrient application in the seed stage contributes to an early and productive development of crops. Since silicon (Si), as a nutrient for plants, acts in the cellular structure and the formation and performance of plant architecture, the aim of this study was to evaluate the influence of silicon application via seeds on the initial growth of three soybean cultivars. The experimental design was a randomized block. The treatments were replicated three times and distributed in a 3×5 factorial scheme, composed by three soybean cultivars (C1 - FTR 1186 IPRO, C2 - FTR 1192 IPRO and C3 - FTR 3190 IPRO) and five silicon doses (0, 30, 60, 90 and 120 g per 100 kg of seeds). The data were submitted to ANOVA by F-test and polynomial regression analysis for the silicon doses; the cultivars response was evaluated through the Tukey mean test. The treatment of soybean seeds with silicon did not positively influence the root dry mass of the FTR 1192 IPRO, nor the root length and root diameter of the FTR 1186 IPRO. The application of silicon did not influence the FTR 3190 IPRO.

Keywords:
Beneficial nutrient
Mineral fertilization
Seed treatment
Silicon

RESUMEN

La soya (*Glycine max*) es un cultivo de gran poder económico en el mundo, siendo destinada a la producción de aceite vegetal, así como para la alimentación animal y humana. Investigaciones recientes indican que la aplicación de nutrientes en la fase de semilla contribuye al desarrollo inicial y productivo de los cultivos. Dado que el silicio (Si), como nutriente para las plantas, actúa en la estructura celular y en la formación y el desempeño de la arquitectura de la planta, el objetivo de este estudio fue evaluar la influencia de la aplicación de silicio a través de las semillas en el crecimiento inicial de tres cultivares de soya. El diseño experimental fue de bloques aleatorios. Los tratamientos fueron replicados tres veces y se distribuyeron en un esquema factorial 3×5, compuesto por 3 cultivares (FTR 1186 IPRO, FTR 1192 IPRO, FTR 3190 IPRO) y cinco dosis de silicio (0, 30, 60, 90 y 120 g por 100 kg de semillas). Los datos fueron sometidos al análisis de varianza por la prueba F y regresión polinomial para las dosificaciones de silicio, la respuesta de los cultivares fue evaluada por medio de la prueba de medias de Tukey. El tratamiento de las semillas de soya con silicio no influyó positivamente en la masa seca de la raíz del cultivar FTR 1192 IPRO, ni en la longitud y el diámetro de la raíz del cultivar FTR 1186 IPRO. El cultivar FTR 3190 IPRO no fue influenciada por la aplicación de silicio.

Palabras clave:
Elemento benéfico
Fertilización mineral
Tratamiento de semillas
Silicio

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The soybean [*Glycine max* (L.) Merrill] is the most important crop cultivated in Brazil. During the period 2015 – 2016, the country's total production was 95.63 million tons, produced on 33.17 million hectares (Embrapa, 2016). Nowadays, the soybean crop is largely cultivated and consumed worldwide, being an important agricultural product destined for animal feed and human food, thus highly demanded in the agrobusiness world (Conab, 2016).

High-quality seeds are necessary to obtain an optimal seedling population. High physiological quality soybean seeds will grow into plants with greater morphological and biochemical performance, thereby increasing the chance of better development and yield (Kolchinski *et al*., 2005). Brazilian agricultural researchers have developed new soybean cultivars with expressive yield potential, substantiating the crop importance in the international market and export trade.

In the agricultural production system, it is a challenge to uniformly distribute, in the soil, the nutrients that are required in small amounts by the plants. Seed treatments have enabled plants to have higher performance and full genetic potential achievement through the application of pesticides, biological products, plants stimulants, and inoculants, as well as the use of micronutrients, such as silicon (Daronco, 2013). In this sense, seed treatment might be an alternative to maximize agricultural yield (Oliveira *et al*., 2013), making it necessary the use of techniques directed to improve legume plants mineral nutrition.

The nutritional essentiality of silicon is controversial. Some researchers claim it is essential to plants, while others refer to it as a beneficial nutrient. Some studies concerning silicon have revealed increasing in the physical and structural resistance against biotic and abiotic factors in several species caused by an increase in the cell wall, middle lamella thickness, and rigidity, because of the application of silicon with phenolic compounds and pectins (Datnoff *et al*., 2007; Currie and Perry, 2007). Moreover, silicon might reduce aluminum toxicity, as well as other micronutrients toxicity, such as manganese, iron, and sodium (Castro and Crusciol, 2013).

The potassium silicate (K_2SiO_3) fertilization brings several benefits to the soybean crop. It makes leaves more upright, decreasing the plants self-shading. It also enhances the tolerance to lodging and the plants' resistance to several stresses through the accumulation of silicon in their tissues. Also, potassium influences stomatal activity and improve gaseous exchanges in plants while silicon can protect them from pathogens and phytophagous insects, boosting the plants' development and yield (Wise *et al*., 2007; Zelin *et al*., 2011).

Machado and Queiroz (2018) reported that the treatment of soybean seeds with silicon increases the vigor of the seeds under laboratory conditions, but the agronomic characteristics in the field were not influenced. Oliveira *et al*. (2015) also pointed out that the application of silicon does not impair the physiological quality of soybean seeds. According to these authors, the agronomic characteristics of the cultivars are not influenced by the doses of silicon, as well as the number of vegetables per plant. Foliar application of potassium silicate in maize, enhanced the plants' photosynthetic efficiency (Sousa *et al*., 2010). Ferreira (2008) verified positive effects on the photosynthesis, transpiration rate, stomatal conductance and internal CO_2 concentration of cotton plants treated with sodium metasilicate solution. In this sense, the aim of this study was to evaluate the influence of silicon application via seeds on the initial growth of three soybean cultivars (*Glycine max* L.).

MATERIALS AND METHODS

The study was carried out from October to November 2016, at the greenhouse of the Center of Agricultural Sciences, in the Universidade Federal de Paraíba, located in the city of Areia, state of Paraíba, Brazil. According to the climate classification of Köppen-Geiger, the As' climate (hot and humid) is observed in the region, which presents a rainy season concentrated between March and July and an average annual rainfall of 1400 mm (Peel *et al*., 2007).

The experimental design was a randomized block, and the treatments were replicated three times and distributed in a 3×5 factorial scheme, composed by three soybean cultivars (C1 - FTR 1186 IPRO, C2 - FTR 1192 IPRO and C3 - FTR 3190 IPRO) and five silicon doses (0, 30, 60, 90 and 120 g per 100 kg of seeds). The silicon doses were applied via seeds according to the proposed by Oliveira *et al*. (2014). The silicon doses were prepared with liquid potassium silicate (K_2SiO_3), which presented

the following composition: 130 g L^{-1} of SiO_2 and 130 g L^{-1} of K_2O. The potassium silicate was directly applied in 100 g seed in the following proportion: 0, 230, 460, 690, and 920 mL K_2SiO_3 100 kg $seeds^{-1}$, equivalent to the silicon doses determined for the experiment. The seeds were immersed in the solution for 12 h.

The soil used was classified as Haplic Planossoil (Embrapa, 2014), collected in the 0-30 cm depth, from the lands of the Center of Agricultural Sciences, in the Universidade Federal de Paraíba. The physicochemical characteristics of the soil are shown in Table 1. The experimental units were 5-liter pots, where the soybeans were cultivated. The soybean seeds were treated with the fungicide thiophanate-methyl (5 g a.i. per 100 kg of seeds) and the insecticide fipronil (50 g a.i. per 100 kg of seeds). The fertilization was done according to the recommended to the soybean crop.

Table 1. Physical and chemical soil analysis report of the Haplic Planossoil used in the study.

Depth (cm)	pH H_2O	P (mg dm^{-3})	K^+	Ca^{2+}	Mg^{2+}	Na^+	Al^{3+}	H+Al	BS (%)	TOC (g kg^{-1})
			($cmol_c$ dm^{-3})							
0-10	6.0	0.83	0.02	2.15	0.65	0.02	0.05	3.22	46.9	6.2
10-30	5.8	1.59	0.28	3.35	0.98	0.03	0.05	4.87	48.8	7.0

TOC: Total Organic Carbon; BS: % Base Saturation

In each experimental unit, two seeds were sown at a depth of 1 cm. Following germination, plants were thinned to one plant per pot. The plants were irrigated whenever needed in order to maintain the soil at field capacity. The study was conducted until the V5 phenological stage of soy development, according to the classification of Fehr and Caviness (1977).

The emergence speed index (ESI), plant height (cm), leaf area (cm^2 $plant^{-1}$), stem diameter (mm), shoot dry mass (g), root length (cm), root diameter (mm), and root dry mass (g) were measured. Plant height, leaf area, and root length were measured using a millimeter ruler. Stem and root diameter were measured using a pachymeter. Forced air circulation oven and analytical balance (0.001) were used to determine shoot and root dry mass. The emergence speed index was calculated based on Maguire's formula (Maguire, 1962). The leaf area was measured according to the methodology described by Richter *et al.* (2014).

The data were submitted to ANOVA F-test. Tukey test, at 5% of significant level, was used for the soybean cultivars and polynomial regression analysis for the potassium silicate doses. Data analysis was performed using the SAS software (Cody, 2015).

RESULTS AND DISCUSSION

According to the initial development analyses, the emergence speed index presented no significant effect on the soybean cultivars submitted to different silicon doses. The average emergence speed index was 6.4% (Figure 1). This result might be related to the high-vigor soybean seeds that were used in this study, which led to a faster emergence. Some researchers registered similar results. Pereira *et al.* (2004) found that seed treatment with potassium silicate did not influence the emergence speed index of rice (*Oryza sativa* L.).

In irrigated rice, Vieira *et al.* (2011) observed a decrease in the emergence percentage and the emergence speed index when silicon doses are applied 30 d before sowing. This fact may be due to toxicity generated over extra high calcium silicate doses in the paddy field. These authors stated that highest emergence percentage and emergence speed index were observed in areas that did not receive any calcium silicate dose (0 t ha^{-1}), with an emergence speed index of 3.3 and an emergence percentage of 77%.

Seed treatment with potassium silicate did not influence the soybean plants' height. However, differences were observed between the soybean cultivars (Figure 2). The FTR 1192 IPRO (C2) cultivar presented the highest height, differing only from the FTR 3190 IPRO (C3) cultivar, with mean values of 28.5 and 24.8 cm, respectively. The FTR 1186 IPRO (C1) cultivar average height was 26.7 cm. The FTR 1192 IPRO cultivar has a high potential for rooting and rusticity which may have

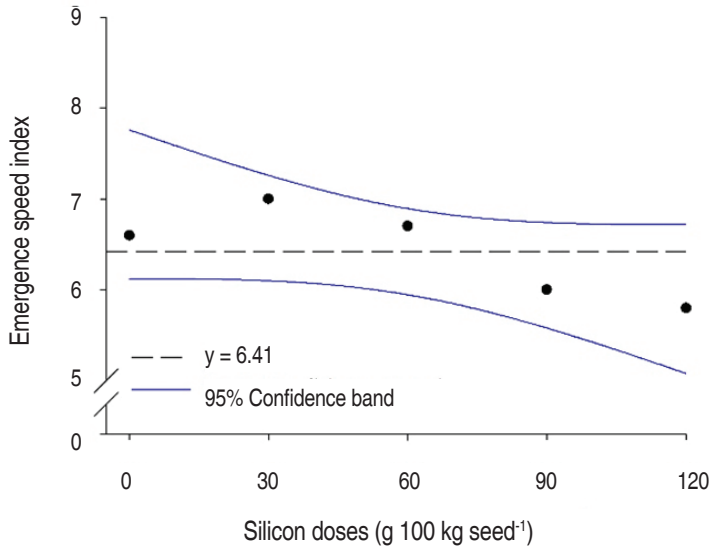

Figure 1. Emergence speed index of soybean seedlings submitted to silicon fertilization via seeds.

favored its prominence concerning the FTR 3190 IPRO cultivar.

Oliveira *et al*. (2015) studied the effect of seed treatment with different doses of silicon on the height of two soybean genotypes. The silicon treatment did not influence the height of BMX Turbo RR and NA 5909 RR genotypes while plants that received no silicon application reached greater heights. Still, Pereira Júnior (2008) registered higher heights in soybean plants treated with silicon, contrasting the results mentioned above. The BRS MG 68 cultivar presented average height of 91, 96 and 94 cm when treated with 400, 450 and 500 kg Si ha^{-1}, respectively.

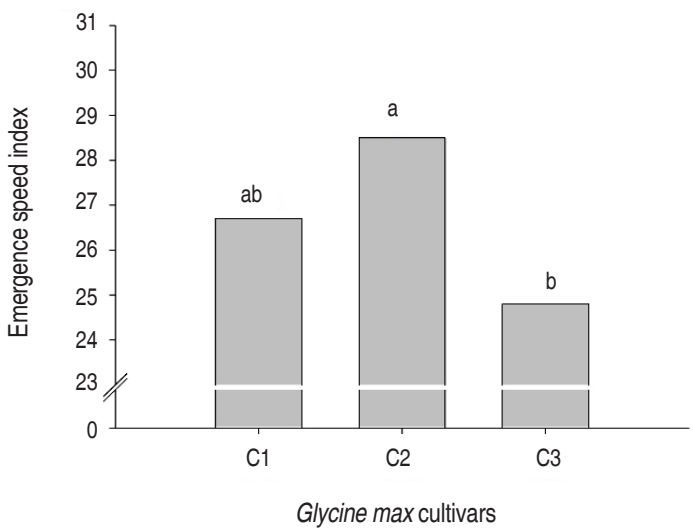

Figure 2. Plant height of three soybean cultivars submitted to silicon fertilization via seeds. Means followed by the same letter do not differ significantly (α=0.05)

Regarding the leaf area, seed treatment with different doses of potassium silicate did not influence the soybean cultivars (Figure 3). According to Takahashi *et al.* (1990), legume plants usually accumulate low levels of silicon in its cellular structures because they consume silicon in favor of a transpiration flow, which is a slower process when it is compared to water absorption. Besides, silicon is not diffused in the symplastic pathway.

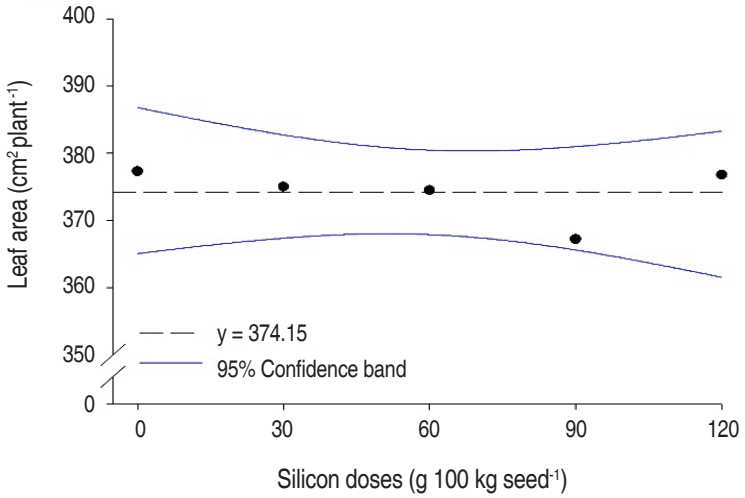

Figure 3. Leaf area of soybean plants submitted to silicon fertilization via seeds.

Comparable results to the ones found in this study were observed by Oliveira *et al.* (2015) that emphasize that the morphological characteristics of the soybean varieties BMX Turbo RR and NA 5909 RR are not influenced with the doses of silicon via seed treatment. Agarie *et al.* (1998) stated that silicon might be associated with leaf retention because of photosynthesis maintenance and chlorophyll distribution under stress conditions, such as high temperatures and low humidity. This remark might have influenced the greater leaf area and dry mass accumulation in the soybean plants submitted to silicon fertilization at the phenological stages mentioned above.

Silicon is deposited in the plants' structure through root or foliar uptake. It stabilizes in the form of hydrated amorphous silica (SiO_2nH_2O) with polysaccharides (cellulose and hemicellulose) on cell walls. The presence of silicon makes the leaves more upright, which enhances CO_2 uptake, as well as solar radiation exposure. As a result, it might improve soybean photo-assimilates production, directly influencing the crops initial development and yield (Epstein, 1999).

Regarding the stem diameter, potassium silicate treatments did not influence the studied soybean cultivars (Figure 4). However, several authors registered higher stem diameters in maize plants submitted to silicon fertilization (Neri *et al.*, 2009). These increases are probably related to the monocots plants ability to accumulate more silicon through an active uptake process substantially. The silicon accumulates in the lumen and intercellular spaces of Poaceae plants leaves and culms, such as maize and sugar cane (Epstein, 1999).

When studying the effect of micronutrients fertilization and seed inoculants on BRS 206 soybean cultivar, Souza *et al.* (2009) registered higher stem diameter values compared to the ones found in this study. However, no significant effect was observed between the applied treatments. The higher stem diameter values found by other researchers is related to the different soybean genotypes studied and different phenological stages evaluated.

Regarding the dry shoot mass, significant effect was observed for FTR 1192 IPRO (C2) cultivar, in response to the different doses of potassium silicate applied. The dry shoot mass of C2 cultivar adjusted to the quadratic model, presenting the higher value, approximately 2.1 g, when 120 g of silicon was applied (Figure 5). Because of the greater rooting, when compared with the others, the C2 variety

responded better concerning the increase of dry mass. It may be linked to the fact that with a more developed root system the plant can absorb more nutrients and, as a result, develop better.

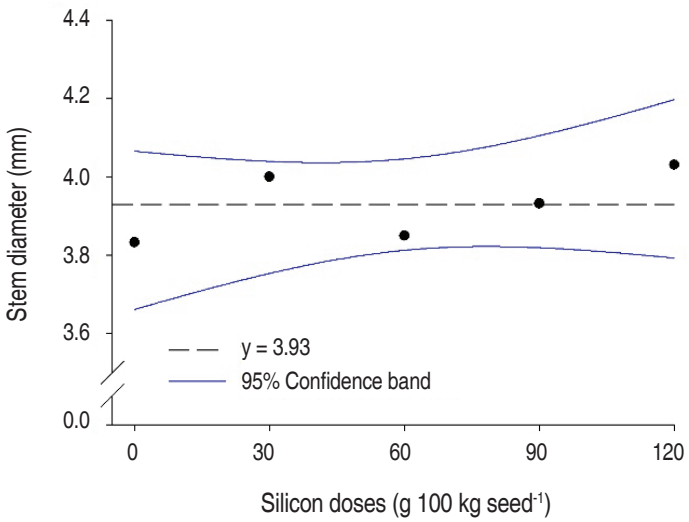

Figure 4. Stem diameter of soybean plants submitted to silicon fertilization via seeds.

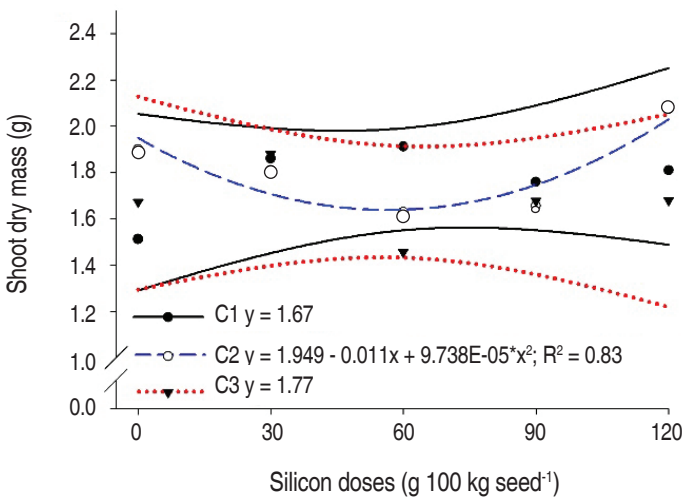

Figure 5. Shoot dry mass of soybean plants submitted to silicon fertilization via seeds.

Sousa *et al*. (2010) verified significant variations in the dry culm mass of maize plants fertilized with K_2SiO_3. Soratto *et al*. (2012) and Figueiredo *et al*. (2007) reported higher shoot dry mass of strawberry and potato plants fertilized with silicon, respectively. Teodoro *et al*. (2015) studied the K_2SiO_3 foliar fertilization effect in the soybean cultivar 5DR615 and verified greater total dry mass production in the crop R6 phenological stage.

Regarding the root parameters, FTR 1186 IPRO (C1) cultivar root length and diameter linearly increased as the K_2SiO_3 doses increased (Figures 6 and 7). The C1 cultivar average values for root length and diameter when treated with 120 g of silicon were 8.7 cm and 4.5 mm, respectively. The FTR 1192 IPRO and FTR 3190 IPRO cultivars root length and diameter presented no significant results in response to the silicon treatment.

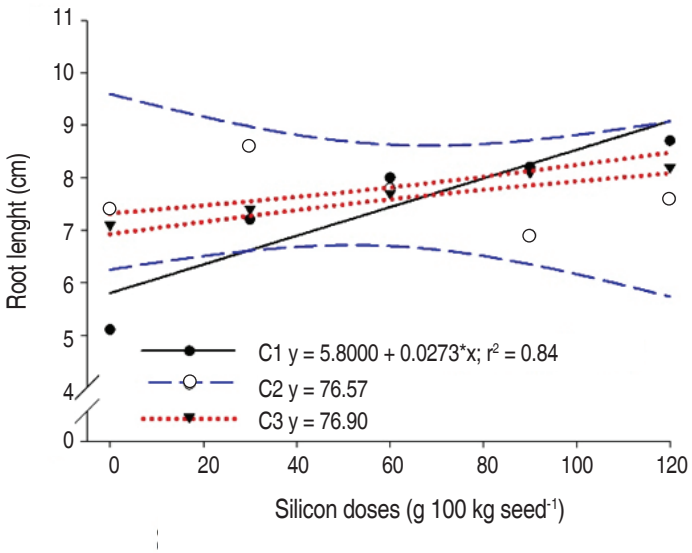

Figure 6. Root length of soybean cultivars submitted to silicon fertilization via seeds.

The results mentioned above verified for the C1 cultivar might be related to the SiO_2 accumulation by the roots. Silicon-non-accumulative species are unable to accumulate and translocate high levels of silicon in their shoots; thus they tend to concentrate this element in the root system, where most of it is deposited in the cell walls (Heine *et al.*, 2005). Soybean plants translocate low levels of silicon to the shoots, up to 30 mg kg^{-1}. This substantiates the fact that in legume plants, such as soybean and bean plant, there is greater silica (SiO_2) accumulation in the roots.

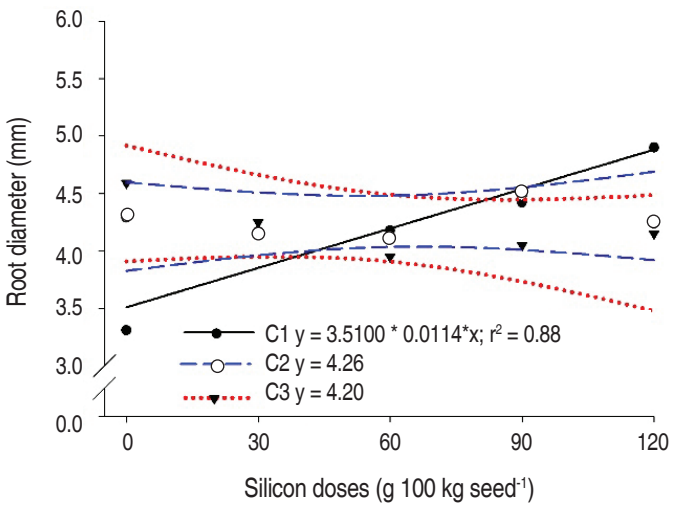

Figure 7. Root diameter of soybean cultivars submitted to silicon fertilization via seeds.

Silicon absorption is mediated by diffusion in soybean and bean plants, through the movement of mono-silicic acid from a high to a low concentration region. This transport process may require energy, due to the silicon's translocation across the water channel proteins, the aquaporins. In this process, silicon molecules retention by the suberin layer present in the root endodermis can occur, making the roots thicker (Raven, 2001).

Silicon is an important element for the soybeans root system, once enhanced nodulation and nitrogen fixation is observed in silicon fertilized soybean crops (Figueiras, 2007). However, some factors must be observed before applying silicon fertilization, such as the genetic characteristics of the cultivar, its ability to consume, translocate and accumulate the element, as well as the soil and environment conditions.

The silicon doses did not influence the roots' dry mass but the different soybean cultivars presented statistical differences among themselves. The FTR 1192 IPRO (C2) cultivar presented the highest root dry mass compared to the other studied cultivars (Figure 8). These results reveal that same species plants (*G. max*) genotypes, presented different root dry mass accumulation patterns.

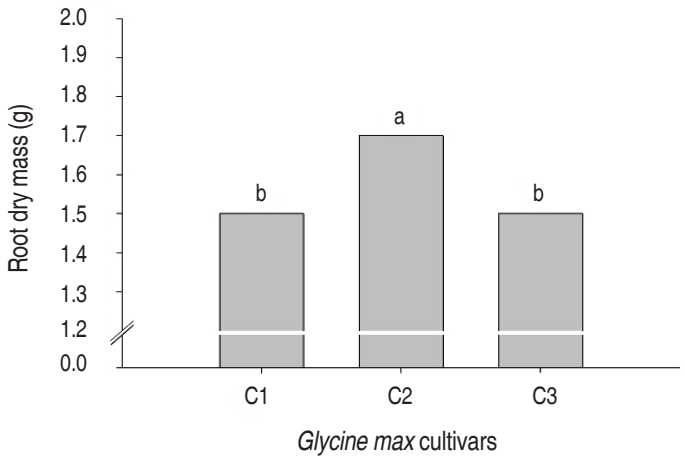

Figure 8. Root dry mass of soybean cultivars submitted to silicon fertilization via seeds. Means followed by the same letter do not differ significantly (α=0.05).

Paula *et al*. (2007) reported higher roots dry mass in fava d'anta plants (*Demorphandra mollis* Benth) submitted to potassium silicate fertilization. Still, Ribeiro *et al*. (2011) verified limited root dry mass accumulation in coffee plants submitted to silicon fertilization after 130 of treatment.

CONCLUSIONS

The treatment of soybean seeds with silicon did not positively influence the root dry mass of the FTR 1192 IPRO variety and the root length and root diameter of the FTR 1186 IPRO variety. The application of silicon did not influence the FTR 3190 IPRO cultivar. Regarding the plant height and dry root mass parameters, the FTR 1192 IPRO cultivar presented more precocity compared to the other genotypes.

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Characterization, performance and level of technology adoption of the plantain agro-systems in Antioquia, Colombia

Caracterización, desempeño y nivel de adopción tecnológica de los agro-sistemas plataneros en Antioquia, Colombia

doi: 10.15446/rfnam.v72n2.69897

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ABSTRACT

The aim of this research was to identify and evaluate socioeconomic and agronomic indicators of plantain agro-systems in 14 municipalities located in the Southwest and Urabá subregions of Antioquia. These subregions present high commitment on the plantain crop, but with different levels in its management and trade. In order to address this research 197 socio-agronomic surveys were conducted; the survey was composed by 93 questions covering the following topics: general information, sources of income, plantain crop management, marketing of the fruit and environmental management. It was found that the production of Dominico Harton in the Southwest varies between 3 and 5 t year^{-1}. On the other hand, it was observed that 84% of the plots established with plantain crops are classified as small producers, with areas sown under 5 ha. In addition, it was determined that the farms that sold to traders, in general, presented higher gross income than those who offer to collection centers. The production of Harton in Urabá was between 7 and 23 t year^{-1}. The lands with the highest income were those who sold their products to traders or by direct sales in the modalities fruit in cases or fruit infield, respectively. In both sub-regions, the largest proportion of the area established in plantain corresponds to the premises of peasant economy, small producers that till for the agronomic maintenance of the crops. However, the yields presented in the Southwest subregion suggest the necessity for higher technical intervention in the crop.

Keywords:
Agronomic management
Musa AAB Simmonds
Socioeconomic indicators
Trading

RESUMEN

El objetivo de este trabajo fue identificar y evaluar indicadores socioeconómicos y agronómicos de agro-sistemas plataneros en 14 municipios localizados en las subregiones del Suroeste y Urabá antioqueño. Subregiones con alta vocación en el cultivo de plátano, pero con diferentes niveles de manejo y comercio. Para abordar esta investigación se realizaron 197 encuestas de carácter socio-agronómico; la encuesta estuvo conformada por 93 preguntas comprendidas en los siguientes temas: información general, fuentes de ingreso, manejo del cultivo, comercialización de la fruta y manejo ambiental. Se encontró que la producción de plátano Dominico Hartón en el Suroeste varía entre 3 y 5 t año^{-1}. Por otra parte, se pudo observar que el 84% de los lotes establecidos con cultivos de plátano se clasifican como pequeños productores, con áreas sembradas menores a 5 ha. Además, se determinó que los predios que venden a comercializadoras presentan, en general, mayor ingreso bruto que aquellos que los ofrecen a centros de acopio. La producción de Hartón en el Urabá estuvo entre 7 y 23 t año^{-1}. Los predios con mayor ingreso bruto fueron aquellos que vendieron su producto a comercializadoras o por ventas directas en las modalidades de fruta en caja o fruta desmanada en campo. En ambas subregiones, la mayor proporción del área establecida al cultivo plátano corresponde a predios de economía campesina, pequeños productores que laboran para el mantenimiento de los cultivos. Sin embargo, los rendimientos presentados en la subregión del Suroeste sugieren la necesidad de una mayor intervención técnica en el cultivo.

Palabras clave:
Manejo agronómico
Musa AAB Simmonds
Indicadores socioeconómicos
Comercialización

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It is estimated that 87% of the cultivated area in Colombia correspond to traditional crop management associated with coffee, cocoa, cassava, and fruit trees; the remaining 13% correspond to modernized monocultures. The cultivation of plantains in Colombia has been a traditional sector of peasant economy, subsistence for small producers, high geographical dispersion and high socioeconomic importance for the food safety and employment generation. Close to 4% of the national production of plantains is intended to be part of the export market, the remaining fruit is intended for fresh domestic consumption, and less than 1% is used as a source of raw material for the national agroindustry (Espinal *et al*., 2005). The agricultural sector is composed of small producers that work on the available lands of Colombia which are a fixed resource. However, the problem in productivity is associated with additional factors, such as technology, good agricultural practices, inputs, the educational level of the producer, technical assistance, among other factors, which allow greater production per unit area.

In order of importance, the departments that are producing the largest volumes of fruits in Colombia are Quindío, Meta, Antioquia, and Tolima. Among the varieties that are sold the most in plantain regions of Colombia are Harton, Dominico Harton, and Dominico, which are usually eaten fresh, either green or ripe; it is also possible to obtain from them alcohol, flour, wine, starch, snacks or nutritional supplements for animals. Currently, the plantain cultivation has grown dramatically in the department of Antioquia. According to Agronet (2016), the area raised with plantain in Antioquia was 62,686 ha, from which 57,019 were harvested, presenting a production of 457,363 t and a yield of 8.02 t ha^{-1}. The commercial plantations of high and low density of planting are classified, according to the amount of hectares that composed them, in business (>30 ha), large (15.1-30 ha), medium (5.1-15 ha) and small (0.1-5 ha) (Meek and Aldana, 2001; Roldán *et al*., 2004). Additionally, in the department of Caldas, it was identified that most of the plantain producers are smallholders (between 1 and 10 ha) (León-Agatón *et al*., 2015).

In Antioquia, the plantains are produced mainly in the subregions of Southwest (as an associated crop to coffee) and Urabá (as a clean crop - monoculture). In 2007, the Southwest presented a total area in plantain of 11,491 ha, a production volume of 52.13 t, and a performance average of 4,536 kg ha^{-1}. Urabá for its part, had a total area of 36,845 ha, a production volume of 394 t, and an average yield of 10,673 kg ha^{-1} (SIC, 2012). After the coffee crops, the plantain is the agricultural line of major economic importance in the Southwest. It has traditionally been used as a shade tree for shade-grown coffee, and its production is intended for internal consumption in farms, especially in times of coffee harvest when the migrant population increases. Plantain crop also represents in Southwest surplus, especially in times of plenty harvest (Castro *et al*., 2009). Following the above, the plantain crop is not the main product in the economy of the Southwest subregion, being the Jericó municipality the one with the highest yield (8 t ha^{-1}) (Agronet, 2016). On the other hand, the subregion of Urabá excels by the degree of specialization that it has reached in the production and export of banana and plantain. Its highest yields are due to the high levels of productivity, the integration of producers and traders, the comparative advantages of location, and the quality of its soils in comparison with other producing areas in the world (Espinal *et al*., 2005); according to Agronet (2016), Turbo is the municipality with the highest yield in the Urabá subregion with a yield of 10.7 t ha^{-1}.

According to SIC (2012), there are different actors involved in the productive plantain chain affecting their quote, such as the producers that perform the agronomic management during the production cycle until his harvest. The distributors and suppliers are responsible for the grading and packing, taking the appearance, size, and quality of the fruit into account. The conveyor of fruit has an important role in its transportation. Finally, the wholesaler addresses the distribution to the various channels, where the price is determined in the destination. With that being said, the objective of this work was to characterize, according to the yields and the level of technology adoption of the producers, the first actors of the plantain product chain of the Southwest and Urabá subregions in Antioquia, Colombia.

MATERIALS AND METHODS

Area of study

This project was carried out in the Southwest and Urabá subregions of Antioquia, Colombia. The Southwest

subregion lies between 800 and 1800 m of altitude (Castro *et al*., 2009). This region presents a moderately high rainfall that exceeds the potential evapotranspiration. It presents average temperatures without seasonal variation and absence of frost, with a multi-year average value of 20 °C and approximate limits between 17 and 24 °C, showing conditions of a lower montane wet forest. On the other hand, the Urabá subregion is dominated by a gradient of humidity, which increases from the driest municipalities of Arboletes and San Juan de Urabá, located to the north of the Department, to the municipality of Mutatá in the south of the region. This gradient has established several life zones considered in the studied area of the following municipalities Arboletes and San Juan de Urabá (tropical dry forest), San Pedro de Uraba and Turbo (tropical moist forest), Necoclí (premontane moist Forest), and Mutatá (tropical wet forests) (García *et al*., 2007).

Implementation of the social-agronomic survey in the chosen municipalities

A participatory diagnosis was carried out through the socio-agronomic survey. In the Southwest subregion the municipalities that were chosen to be evaluated were Andes, Betania, Hispania, Jardin, Jericó, Pueblo Rico, and Támesis; from the Urabá subregion, the chosen municipalities were Arboletes, Mutatá, Necoclí, San Juan de Urabá, San Pedro de Urabá, and Turbo. The respondents from both subregions were invited to participate in the survey by the Secretary of Agriculture, Farmer's Associations, and Cooperatives. 62 smallholder producers from Southwest and 135 from Urabá attended to the invitation. The socio-agronomic survey consisted of 93 questions, grouped into the following topics: General information of the respondent, sources of income, agronomic management of the plantain crop (plant-harvest-post-harvest), ways of trade the fruit and environmental management of the crop. The logistical support to perform the survey was carried out by the Secretary of Agriculture and Environment of each municipality, the Cooperativa Sanbartolo (COMSAB), the Banana Association of Colombia (AUGURA), the International Merchants Banacol, Union of Banana Plantations of Urabá (Uniban) and The Association of Plantain Producers of San Juan de Urabá.

Analysis of the information

The data obtained were tabulated and summarized in worksheets and the corresponding descriptive analysis was carried out with the software R-project version 3.2.1 (R Core Team, 2016).

RESULTS AND DISCUSSION

Characterization of the Southwest subregion

Established area for plantain crops. Southwest had a total plantain area of 11,491 ha in 2007 (Castro *et al*., 2009). Through the survey, it was found that most of the farms established for plantain crops (associated with coffee) had an area between 1 and 2 ha (47%), a few farms (8%) extended an area between 2 and 3 ha. Nearly the same proportion of farms presented less than 1 ha (11%), between 3 and 5 ha and over 5 ha (18%) (Figure 1). In the study conducted by Meek and Aldana (2001) in Colombia, 80% of the sites correspond to smallholder producers a similar situation was found in Southwest (84%); regarding the medium size producers, the proportion yields up to 15-16% for Colombia and in the Southwest subregion. In contrast to what has been reported for a national scope 5% of business producers (Meek and Aldana, 2001), in the Southwest were not found any producer in the business category (Figure 1).

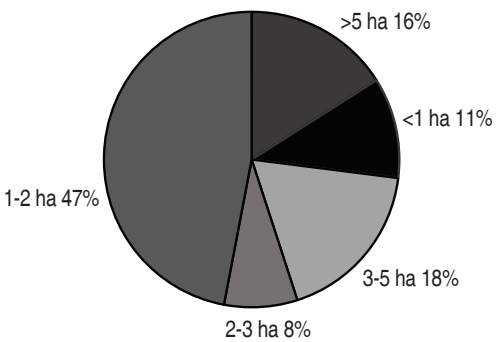

Figure 1. Distribution of the area established for plantain crops in the Southwest of Antioquia.

Plantain yields. According to the data obtained (Table 1), the production of plantains in the Southwest of Antioquia is between 5.34 and 3.32 t ha^{-1} year^{-1}. The 5.5% of the farms categorized with a weight of the plantain bunch greater than 25 kg showed a low production (3.3 t ha^{-1} year^{-1}). On the other hand, 14.5% of the farms were categorized in a cluster of 10 to 15 kg with a production of 3.4 t ha^{-1} year^{-1}. 47.3% of the farms exhibited the highest yields, 5.3 y 4.9 t ha^{-1} year^{-1} with bunches weight of 20 to 25 kg and 15 to 20 kg, respectively. In 2007, this subregion had a production volume of 52.13 t year^{-1}, where the largest producers of plantains on systems associated with coffee were the municipalities of Andes (18.6 t), Abejorral (8.1 t), Támesis (5.8 t), Concordia (3.6 t) and Cocorná (3.1 t), which showed an average yield of 4,669 kg ha^{-1} (Castro *et al.*, 2009), a similar report to the results of production of the Southwest determined in this study, with 4,722 kg ha^{-1}, varying between 3,689 and 5,754 kg ha^{-1} (α=0.05).

It can be inferred that more than half of the production of the farms studied was low (51%, quadrants A and D) and only 14.5% of the study population presented a high weight of bunches and a high production of plantains per year (quadrant B), with a production above the average of 223.6 clusters ha^{-1} (Figure 2).

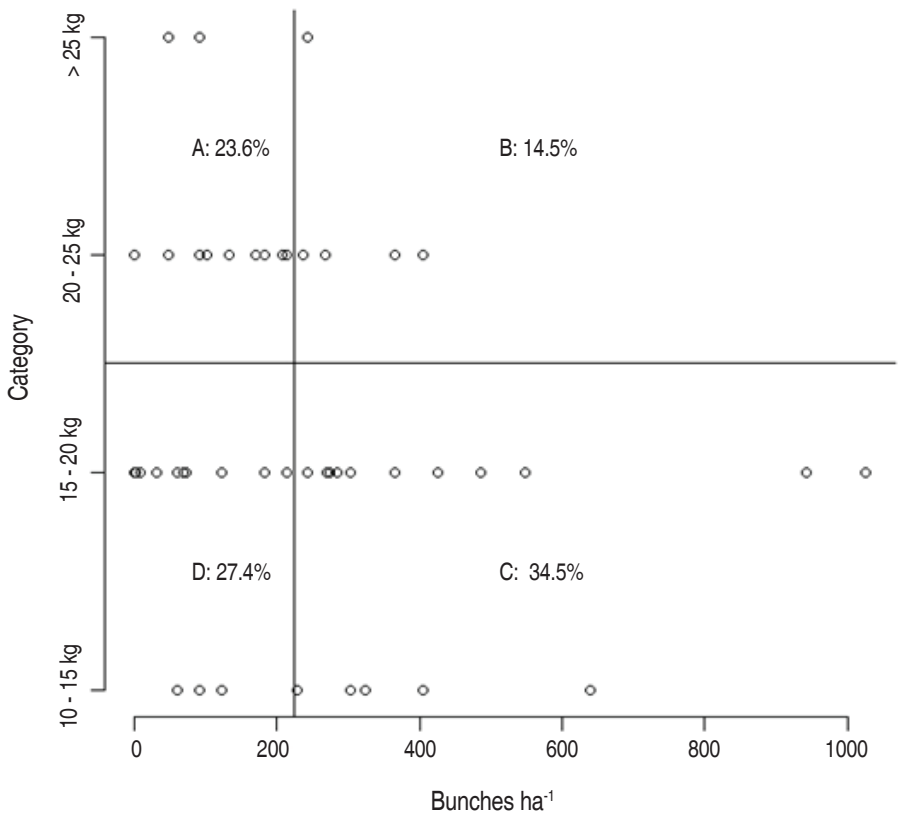

Figure 2. Distribution of producers in the Southwest of Antioquia according to the weight of the bunch of plantains and the number of bunches produced per year. A. Sites with high weight of the bunch and low production; B. Sites with high weight of bunches and high production; C. Sites with low weight and high production; D. Sites with low a weight of the bunch and low production.

Technology adoption. According to the observed data, the adoption of technology for the management of the plantain crops in the subregion is appropriate, except for the bagging. The other agronomic work exceeds 75% of the application. 5.5% of the sites presented a bunch weight greater than 25 kg applied the agronomic work recommended for the crop, 14.5% of the sites obtained a bunch weight between 10-15 kg. The variability

appeared due to the application of the agronomic work in terms of bagging fruit and control of black Sigatoka (*Mycosphaerella fijiensis*) regarding other lands. 80% of producers, with weight of bunches between 15 and 25 kg and higher production, presented a proper application of the agronomic work regarding fertilization, control of black Sigatoka, leaf sheaths removal, thinning, and bagging, represented in greater production (5.14 t ha^{-1} $year^{-1}$ in average) (Table 1). The research carried out by Palencia *et al*. (2006) showed that the most relevant agronomic task for good yields and production of the plantain crops is the control of the weeds, performing fertilizations frequently, thinning, defoliation (control of black Sigatoka), and bagging of the bunch.

Table 1. Percentage distribution of farms and agronomic variables according to the weight of the plantain bunch and its relationship with the annual production for the sub-region Southwest.

Bunch weight (kg)	% P	Agronomic work							EAP (t ha^{-1} $year^{-1}$)
		F	CSTK	LSR	T	B	HC	BC	
		(%)					(d)		
10-15	14.5	87.5	75	87.5	100	50	20	19.3	3.4
15-20	47.3	92.3	96.2	92.3	96.2	88.5	13.81	25.2	4.9
20-25	32.7	77.8	83.3	88.9	94.4	61.1	15.85	32.3	5.3
>25	5.5	100	100	100	100	100	16.13	31.3	3.3

%P: percentage of farms, F: fertilizes, CSTK: controls Sigatoka, LSR: leaf sheaths removal, T: thinning, B: bagging, HC: harvest cycles, BC: number of bunches per crop cycle, EAP: estimated average production.

Distribution and trading channels. The maximum annual income reported in this research was 2,344,614 COP, corresponding with the sites that obtained weight of bunches of 15 to 20 kg. The fruit that is obtained from these sites was sold mostly to collectors (56.5%) and as a second option to traders (34.8%). In contrast, the lands with the lowest income were those that presented a bunch weight over 25 kg, which counted with an annual income of 1,544,761 COP, whose product was sold in larger quantities to traders (66.7%) and as a second option to collectors (33.3%) (Table 2). The farmers, with bunch weight between 15 and 25 kg, direct sale without intermediaries, representing the lowest percentage of participation (25%).

Table 2. Percentage representation of the channel of distribution and presentation according to the weight of the bunch and its relationship with the gross annual income in the Southwest of Antioquia.

Bunch Weight (kg)	Channel of distribution (%)			Trading (%)					GI (COP ha^{-1} $year^{-1}$)
	C	TC	DS	HRPF	HRBF	HRCC	BF	BCC	
>25	33.3	66.7	0.0	0.0	66.7	33.3	0.0	0.0	1,544,761
20-25	50.0	33.3	16.7	0.0	16.7	16.7	55.6	11.1	1,714,105
15-20	56.5	34.8	8.7	13.0	30.5	13.0	43.5	0	2,344,614
10-15	75.0	25.0	0.0	12.5	12.5	25.0	37.5	12.5	1,599,251

C: collector, TC: trading company, DS: direct sales, HRPF: hand removal package infield, HRBF: hand removal boxed infield, HRCC: hand removal in collection center, BF: bunches infield, BCC: bunches in collection center, GI: gross income.

Regarding the way the fruit is traded, most of the farms that obtained higher revenues sold infield. 43.5% in the presentation of bunches and 30.5% hand removal and packed in a box. For the presentation of hand removal and packed in a box; it is important to underline that boxes are packaged by selecting the quality and size of the product. The farms that have the bunch weight above 25 kg do not commercialize the hand removal fruit in bulk infield, bunches infield, or in bunches in the collection center. The 66.7% of sales are carried out infield under the modality of fruit hand removal and packed in boxes and 33.3% through collection centers with the bunch hand removal (Table 2).

According to CCI (2000), there are five distribution channels for bringing the product to the final consumer, among which the most prominent are: Collector - Wholesale - Retailer, Supplier - Wholesale - Supermarket, Producer - Supermarket, Wholesaler - Agroindustry and Producer - Agroindustry; being the first channel the most used. The foregoing is consistent with the observed data, where 54% of the farms sell the product to collectors, 40% to traders (suppliers), and a 6% through direct sales (Producer - Supermarket). In contrast with the income obtained by the sale, the lands that sell their product to supply companies obtain a higher income, compared to the lands that sell to collectors (Table 2, Figure 3).

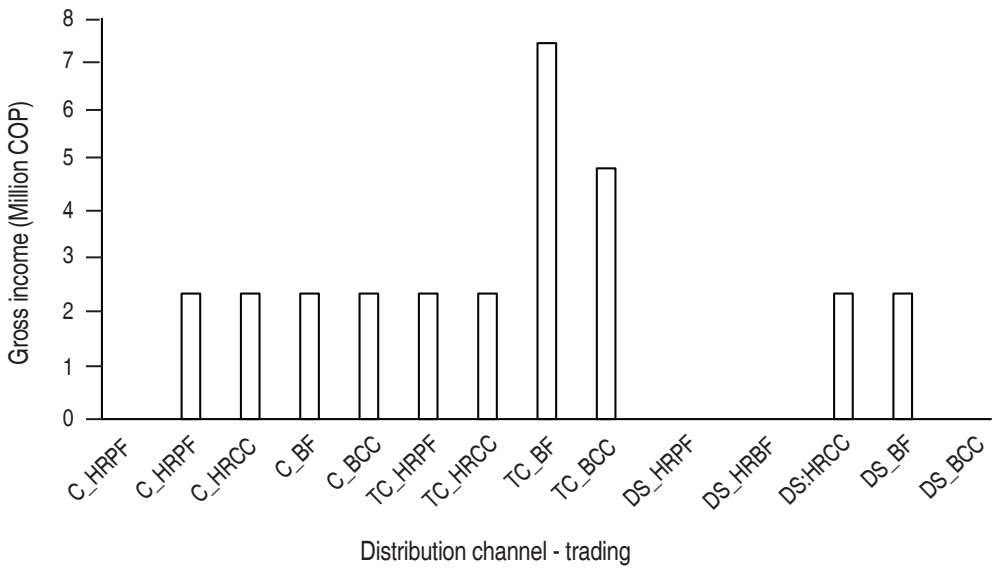

Figure 3. Totals of gross income at sites under different methods of plantain trade in the Southwest subregion.

There are different actors involved and affecting the price of the product such as the producers, distributors, and traders (SIC, 2012). At the same time, the price of plantains is directly affected by factors such as production and harvesting of coffee (Ruíz and Urueña, 2009). In the Southwest of Antioquia, the sale of the product to a Trading Company (TC), in any of the presentations of the fruit, represented a higher income compared with the income accruing for the producers with the other means of distribution, considering that the number of farms that are sold to traders (40%) are less than those who sell to collectors (54%). For the Southwest of Antioquia, the direct sale represents the lower income from the trade of plantains in all their presentations (Figure 3).

Characterization of the Urabá subregion

Established area for plantain crops. In general, it was observed that most of the farms evaluated presented an area destined to the plantain crops less than 5 ha (74%) (Figure 4), which grouped according to the small producers (Meek and Aldana, 2001).

Plantain yields. According to Castro *et al*. (2009), the production of plantains in the Urabá subregion

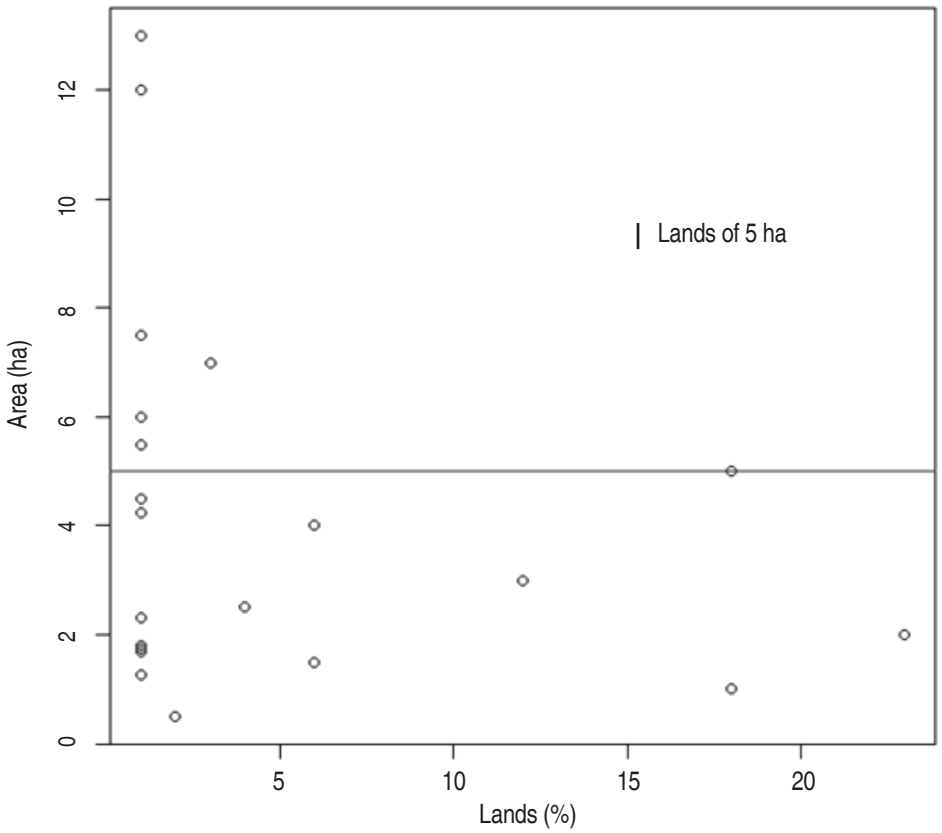

Figure 4. Area set in plantains in Urabá.

was 394 t in 2007, with yields of 10,673 kg ha^{-1}. In the present study, the estimated average production in the Urabá of Antioquia was established in 16,091 kg ha^{-1}. On the other hand, it was observed that 59.3% of the evaluated farms had a bunch weight between 10 and 15 kg; while the lowest amount of land (8.9%) presented a bunch weight between 20 and 25 kg (Table 3).

The productivity of the land is distributed in the majority (61%) above the average of the production of the zone

Table 3. Distribution of farms and agronomic variables according to the bunch of plantains and its relation with the annual production for the Urabá subregion.

Bunch weight (kg)	% P	Agronomic work							EAP (t ha^{-1} year^{-1})
		F	CSTK	LSR	T	B	HC	BC	
		(%)					(d)		
20-25	8.9	90.9	100.0	90.9	100	81.8	9.9	74	23.07
15-20	18.7	95.7	95.7	91.3	100	65.2	11.2	96.7	18.21
10-15	59.3	94.5	95.9	93.2	98.6	74	12.7	102.7	17.80
<10	13.0	93.8	100.0	87.5	100	68.8	11.1	45.9	7.03

%P: percentage of farms, F: fertilizes, CSTK: controls Sigatoka, LSR: leaf sheaths removal, T: thinning, B: bagging, HC: harvest cycles, CC: number of clusters per crop cycle, EAP: estimated average production.

(942.6 bunches of plantains ha^{-1} year^{-1}), which were classified as units of high production of bunches per hectare per year. 11% of the productive units have a high weight of bunches, but a low number of bunches per hectare per year.

17% of the farms obtained greater productivity with high weight of bunches and a high number of bunches per hectare per year while the majority (44%) are efficient in its production, with a low weight of the bunch but with a high number of clusters per hectare per year (Figure 5).

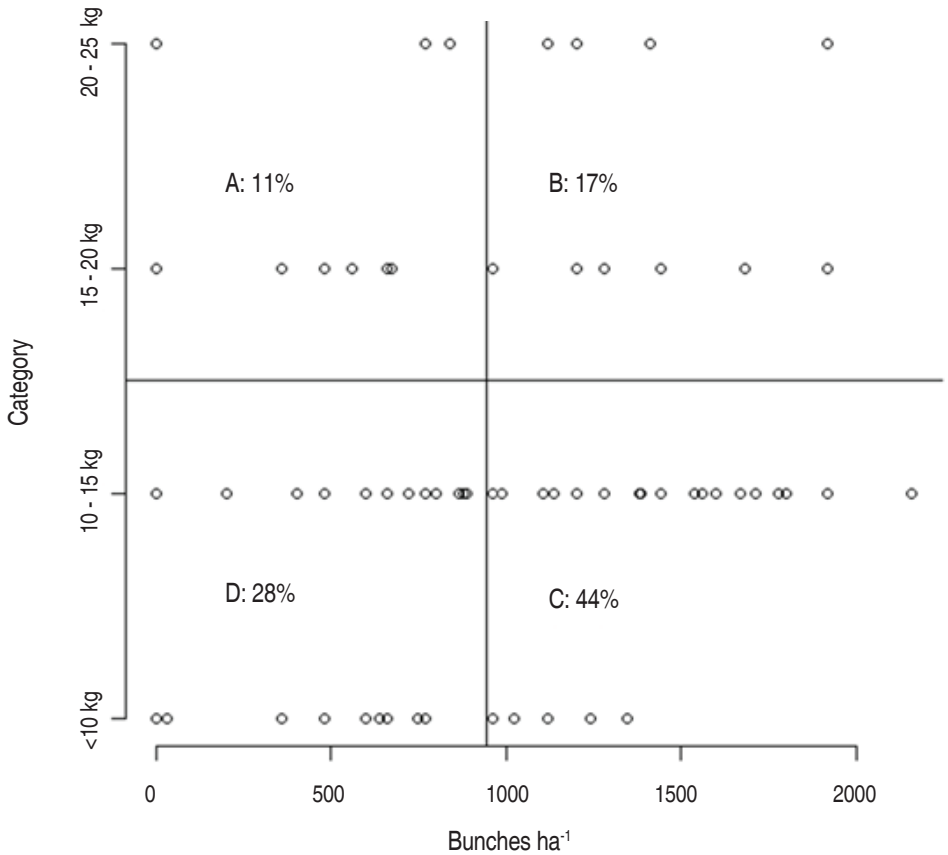

Figure 5. Distribution of producers in the Urabá of Antioquia according to the weight of the bunch of plantains and the number of bunches produced per year. A. Sites with high weight of bunches of plantains and low production; B. Sites with high weight of clusters and high production; C. Sites with low weight and high production; D. Sites with a low weight of the cluster and low production.

Technology Adoption. It was observed that most of the farms, in each of the categories of bunch weight, perform all the agronomic work recommended for the crop; including the bagging of the bunch was the practice less implemented with 72.4% on average (Table 3). Possibly those sites that do not perform all the agronomic work adequately are in the quadrant D, with a smaller number of bunches per hectare and lower weight of bunches (Figure 5). In response to the suggested by Belalcazar Carvajal (1992), who points out that the success of a farm depends on the technology used in the establishment phase and by the class, goodness, and time in which the cultural practices are carried out.

Distribution and trading channels. The largest gross income (10,746,991 COP per year) was observed in producers with bunch weights between 15 and 20 kg. Producers with the lowest weight of bunch (>10 kg) were those who presented the lowest gross income (5,072,666 COP per year), compared with other farmers. In general, most of the sales are made with traders, and the direct sale is the second most chosen option. The

producers with the lowest bunch weight presented the sale with collectors as the second option. The form of selling used by most of the producers was the modality of fruit packed in a box due to it is how the fruit of this region is normally packed and trade, either for the domestic market or for export (Table 4). In comparison with the Southwest subregion, Urabá does not present the modality of selling bunches in the collection center.

Table 4. Percentage representation of the channel of distribution and presentation according to the weight of the bunch and its relationship with the gross annual income in the Urabá of Antioquia.

Bunch Weight (kg)	Channel of distribution (%)			Marketing (%)				GI (COP ha^{-1} year^{-1})
	C	TC	DS	HRPF	HRBF	HRCC	BF	
20-25	10.0	70.0	20.0	0.0	70.0	20.0	10.0	9,106,015
15-20	9.1	59.1	31.8	4.5	63.6	13.6	18.3	10,746,991
10-15	7.0	80.3	12.7	6.8	82.2	1.4	9.6	7,141,270
>10	18.8	68.8	12.5	18.8	68.8	6.3	6.3	5,072,666

C: collector, TC: trading company, DS: direct sales, HRPF: hand removal package infield, HRBF: hand removal boxed infield, HRCC: hand removal in collection center, BF: bunches infield, GI: gross income.

The form of trading had a direct impact on income, as well as the type of customer. The higher revenue obtained in the modalities of fruit packed in boxes and bunch hand removal infield, each one of these with different customers, retailers, and direct sales respectively (Figure 6). According to what has been observed CCI (2000), the distribution channels which registered higher income were Supplier - Wholesale - Supermarket (72.1%) and Producer - Supermarket or Producer - Agro-industry (18%).

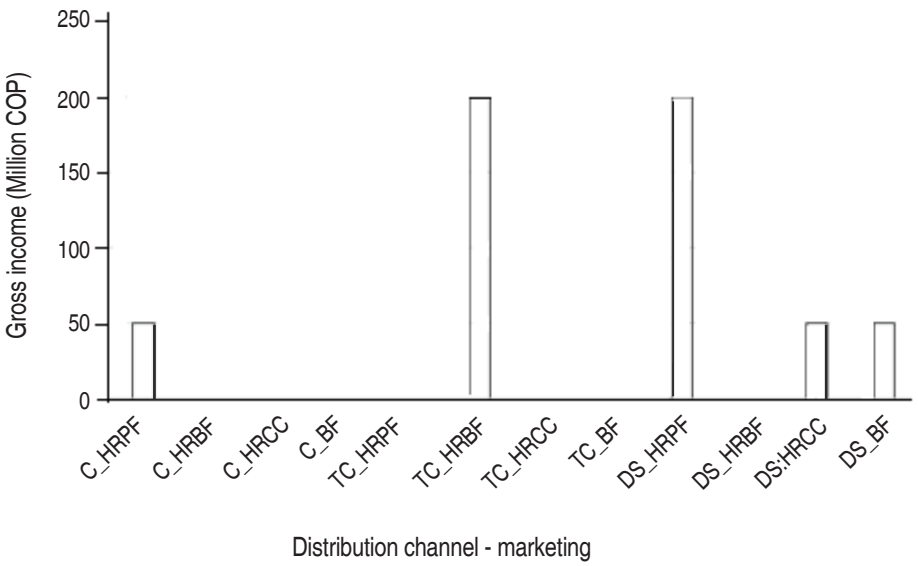

Figure 6. Totals of gross income at sites under different methods of marketing of plantain in the sub-region of Urabá.

CONCLUSIONS

The Southwest of Antioquia has the proper conditions for the agronomic management of the plantain crops, presenting a good production of plantains. 84% of the farms, directed to the plantain crop in the subregion, are classified as smallholder producers, addressing the sale of the plantain to the marketers, instead of to the collector centers. This kind of trade represents a greater gross income for the producers of the subregion. However, it is necessary to implement postharvest methodologies that increase the sale price of the product, such as the break off of the bunch, the packing of the bunch, and the washing of the fruit. The agronomic tasks necessary to carry out the maintenance of the crop are, in general, implemented by the producers in Southwest. The yields obtained in the subregion are linked more to the importance of the crop as the second line in the economy of the subregion. On the other hand, in the Urabá subregion, 74% of the lands intended for the production of plantain compose the peasant economy (<5 ha). The sale to the markets directly represents a higher gross income for Urabá producers, which is in accordance with the sales methodology preferred by them. Plantain production in the subregion is modernized and export-oriented, which is in line with the reported yields and gross income.

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Integral and sustainable community self-management of the native fruit trees of Munhiba, Mozambique

Autogestión comunitaria integral y sostenible de los frutales nativos de Munhiba, Mozambique

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ABSTRACT

With the aim of propose lines of work for the community's appropriate self-management of the native fruit trees in Munhiba, province of Zambézia (Mozambique), it was carried out a study of the perception of the local actors about the causes of deterioration of the fruit trees, as well as their economic, social, and environmental importance. It was used intentional sampling by selecting 118 local actors. In order to develop a Participatory Rapid Diagnosis, exchange workshops and field visits were also performed to identify the fruit species and their use. For the information gathering, semi-structured interviews were applied together with the use of simple and participant observation. Frequency and correlation of variables (Rho of Spearman) were developed for data processing. Among the most important results, it was found the botanical classification of the fruit species, the perception of the local actors about the economic, social, and environmental importance as well as causes of deterioration, identification of strengths and weaknesses for the management of the native fruit trees and a proposal of work lines for their self-management. Despite the potential of native fruit trees in the territory of study, it was not appreciated yet proper management due to the lack of knowledge of the production process that could lead to the progressive deterioration of the species and the ecosystem where they inhabit.

Keywords:
Community self-management
Economic importance
Forest deterioration
Fruit tree species
Mozambique
Working lines

RESUMEN

Con el objetivo de proponer líneas de trabajo para la autogestión comunitaria y manejo adecuado de los frutales nativos en la comunidad de Munhiba, provincia de Zambézia (Mozambique), se realizó un estudio sobre la percepción de los actores locales sobre las causas de deterioro de los frutales, así como la importancia económica social y ambiental. Se realizó un muestreo intencional seleccionando 118 actores locales. En aras de desarrollar un diagnóstico rápido participativo, se realizaron talleres de intercambio y visitas al campo para identificar las especies frutales y usos que se le atribuyen. Para la recolección de información, se aplicó entrevista semi-estructurada junto con el uso de observación simple y participante. Para el tratamiento de datos se usó el análisis de frecuencia y de correlación de variables (Rho de Spearman). Entre los resultados más importantes destacan la clasificación botánica de las especies frutales, la percepción de los actores locales sobre la importancia económica, social, ambiental y las causas de deterioro, se identificaron las fortalezas y debilidades para el manejo de los frutales y se definieron líneas de trabajo para su autogestión. A pesar del potencial de los frutales en el territorio de estudio no se aprecia un manejo adecuado, principalmente por falta de conocimiento en el proceso de producción y recolección de las comunidades locales, lo que puede conducir al deterioro progresivo de las especies y el ecosistema donde habitan.

Palabras clave:
Autogestión comunitaria
Importancia económica
Deterioro del bosque
Especies de frutales
Mozambique
Líneas de trabajo

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Native fruits (NF) in Mozambique are very diverse. Although many of them show broad perspectives of economic use, only a few of them have been studied at both the local and national level. At the same time, the current transfer of scientific and technical knowledge and business opportunities have an incipient value in new productive chains. Besides, a significant part of the indigenous knowledge for the protection of the forest ecosystem has not been considering (Simone, 2001).

The use and logging of the native fruit trees have become into an important practice for the subsistence of rural communities, not only during the seasonal periods of hunger but also in their day-to-day for the nutritious consumption. It must be kept in mind their good adaptability to arid areas without requiring the use of fertilizers, making them more accessible to the rural community, due to their low cost of production and acquisition (Goulão and Santo-Antonio, 2015).

Native fruit trees also play an important role in the traditional medicine and represent a high potential of economic exploitation, in the promotion of employment opportunities and the improvement of the family profit within the rural populations (Chiau, 2003).

Several native fruits, which can contribute to the solution of the alimentary crisis, have been identified within the forest ecosystems in Mozambique. However, the inadequate use and constantly logging are a concern due to their contribution to the decrease in the population of these fruit species, regardless of their importance for the nutritious diet in the Mozambican rural population.

Munhiba is a municipality of the district of Mocuba in which the nutrition security depends on a large part of the production and availability of food coming from a heterogeneous agricultural sector of smallholders, as well as a small incomplete and not well-distributed commercialization network. Agricultural production is characterized by low productivity due to the weak use of technology and incomes. Consequently, the production percentage that reaches the market is low (MAE, 2005).

The low levels of rainfall that have been registered in Munhiba during the last period have placed its population under challenging situations to access to food supplies (nutrition insecurity). Under those conditions, the native fruits have been the fundamental mean of sustenance of local population.

Castro Paulino Comrade, the representative of Food and Agriculture Organization of the United Nations in Mozambique, underlined in the First National Workshop of Native Fruits, that the research in the native area of the fruits in Mozambique is still in its initial phase. He also asserted: “The research, domestication, and development processes of the valuable native fruits chains should deserve a special attention because 70% of the population live in rural areas and almost 80% depend on the natural resources, like fruits and medicinal plants to satisfy the nutritional and health necessities”(Goulão and Santo-Antonio, 2015).

These arguments evidence the necessity to find alternatives to commit the community in the self-management of their natural resources, a subject that has been debated in different trends and socio-political situations along the history of the practical social thinking (Reyes, 2013).

According to Brivio Borja (2001), the community self-management is an administration of the community that occurs due to the transformation of the descending spiral of poverty in an upward spiral of development. The source of the community self-management is the change from a fatalistic vision of the poverty, only as a summary of lacks, to a hopeful vision as a generator of the necessary impulse for development. The community self-management is the channel through which the inherent infinite potential of the human being is canalized toward the achievement of a worthy life, through improving the life quality of each one of the inhabitants. It also allows them to achieve their objectives and goals with solidary support of their fellow men; taking advantage of all the resources of the community, that in this case it is adjusted to the natural resources (native fruit trees).

A worldwide emphasis has been developed toward the managing processes at the community level. Several researchers converge in the topic based on the creation of participative processes that lead to the emergence of a critical popular conscience to understand the social practice as an inseparable unit of reflection and action.

Referring to the case some authors such as Valdés (2010), Mitjans (2012), Sabogal *et al.* (2012), Molero *et al.* (2012), Reyes (2013), and Marzin *et al.* (2014); which offer an updated and in-depth focus on the community actions from the participation and self-management with emphasis in an economic, social, and the environmental scope.

In this research, the self-management is represented as a suitable alternative to the local communities that inhabit the forest ecosystems of Mozambique. They need to be articulated among them to established functions in pro of the forest and their well-being. Therefore, the following hypothesis is presented: if it is considered the economic and environmental perception, as well as the knowledge, that the local actors of the community of Munhiba have about the native fruits of the region; it will be possible to elaborate working lines to contribute with an appropriate management of these native fruits. Regarding the former idea, this research centers its objective in proposing some lines of work for the community of Munhiba that contribute to an appropriate self-management of the native fruits.

MATERIALS AND METHODS

Localization of the study area

Munhiba municipality is located in the central part of Mozambique, in the county of Zambézia, district of Mocuba. It limits to the North with the Headquarters of the District of Mocuba, to the South with the district of Namacurra, to the East with the district of Maganja da Costa trough the Licungo River, and to the West with the town of Namanjavira (MA, 2011).

Methodology used

To assess the economic, environmental, and social perception of the local actors about the native fruits, identify the causes of their deterioration and recognize the strengths and weaknesses for the community development; both quantitative and qualitative research methods were used. Considering the Ethnographic prevalence, the criteria of Rodríguez *et al.* (2008) were used; given that they declared that the study of the Ethnographic is a research method from which it is possible to know the way of life of a specific social unit. Besides, the Participatory Rapid Diagnosis (PRD) was used, following the criteria of Gomes *et al.* (2001) who identify the PRD as the approach most used to facilitate the direct participation of the residents in the generation and the analysis of the obtained information.

For gathering the information, a semi-structured interview along with a simple and participant observation was used to increase the validity of the study and also to obtain a better understanding of the studied context, keeping in mind the approaches proposed by Dewalt and Dewalt (2002).

It was performed an intentional sampling to apply the interview. 118 residents were selected out of a universe of 326 to be interviewed. Three exchange workshops and six field visits were carried out to identify the native fruit species that were in the forest that limits the area where the community inhabits.

For the selection of interviewees, it was taking into consideration those who live up to 2 km of distance away from the forest. The sample size for the research was to determine following the procedures for social studies in finite populations during the estimation of a proportion, presented by Calero Vinelo (1978). A confidence level (1-α) of 95% with a significance level (α) of 5% and a critical value (Z) of 1.96, as well as a positive variability (p) of 0.95 and a negative variability (q) of 0.05 were considered, assuming a maximum permissible error (E) of 0.05.

$$n = \frac{Z^2 pqN}{NE^2 + Z^2 pq}$$

Where:
n: sample size.
Z: level of confidence.
p: positive variability.
q: negative variability.
N: size of the population.

For the interpretation of the interview results, a variable codifier was used (Table 1). The information processing was carried out with the statistical package IBM-SPSS, version 20.0.

For the statistical analysis, non-parametric tests were carried out. It was used Bi-varied correlation using the coefficient of correlation, Rho of Spearman, to determine

Table 1. Variable codifier for the statistical analysis.

Dimensions	Group/Vision		
	1 (Low)	2 (Intermediate)	3 (Advanced)
Knowledge about the identified NF	They know less than 25% of the identified NF	They know between the 25 and 50% of the NF identified	They know 100 % of the identified NF
Perceptions of the NF socio-economic function	They do not consider economic and/or social NF contributions	They consider the NF contributions only to feed	They identify potentialities of the NF as economic and/or social benefits (edible, medicinal, wood, ornamental and other uses like sorcery and fibers)
Perceptions of the NF environmental function	They do not recognize any NF environmental function	They recognize the anti-erosive function of these species	They identify the NF function as a contribution to the soil erosion control and their utility for feeding the fauna
Perceptions about the NF degradation causation	They do not define the causes that have led to the NF degradation	They clearly recognize the responsibility of the NF degradation to the necessity of using these species in wood production	They consider multiple the NF degradation causes, among them: lack of knowledge about the management of these species, wooden use for coal and firewood and the annual fires

(NF=Native fruits)

the strength of the linear relation among the categorical variables. The evaluation of the perception of local actors was also made through a descriptive analysis, based on frequency distributions with the graphics generator.

In order to identify the fruit species taxonomically, there were used not only the criteria of Specialists in Botany and Dendrology from the Agronomy and Forestry Engineering Faculty of Zambeze University, but also the traditional knowledge of the more experienced local actors and the technical record on fruit species proposed by the FAO (1982). Furthermore, an ethnobotanical study was also carried out to assess the strength of the area for the use of the located species. They were classified in: groceries, medicinal, wood, ornamental, sorcery, water store, as well as soil and water protectors.

RESULTS AND DISCUSSION

Perception of local actors on the NF and their relation with the proximity to the forest

During the study of the perception of local actors on the environmental function, it is appreciated that 68 of the interviewees (58%) possess a low vision, because they did not recognize any environmental function of the fruits; out of them, 49 individuals live at a distance between one and two kilometers from the forest. For an advanced vision, 38 individuals were identified, from which 34 live in the forest proximity. In general, it is observed that from the 61 interviewees that live inside the forest area and in its periphery, 40 somehow considered environmental importance of the native fruits, and 19 discarded all environmental value of these species (Figure 1).

Figure 2 shows the relationship between the causes of deterioration and the economic importance that is attributed to the fruit species. It is also appreciated that the largest number of individuals is in the low vision (1) who do not define the causes of deterioration (84/118) (71%), 12 of them do not attribute any benefit, while 72 of them consider its use only as food. So, it can be inferred that if these local actors were qualified about the economic importance of the fruits, they would have been motivated to diminish the causes of deterioration and to self-negotiate the management of the fruits.

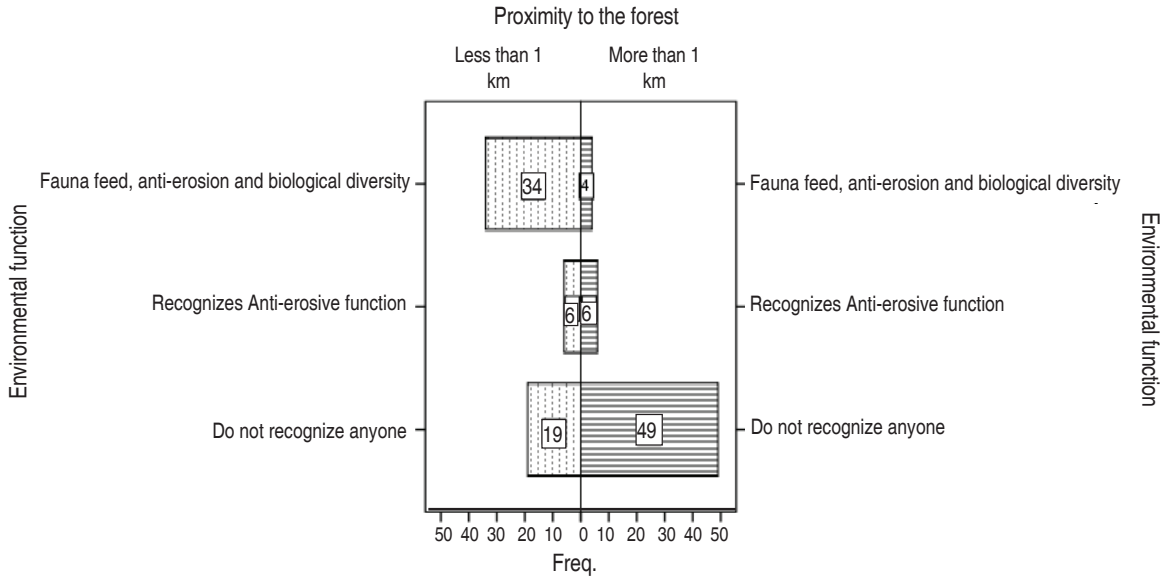

Figure 1. Distribution of frequencies according to the perception of the local actors on the environmental function related to the distance to the forest.

About this concern, AIDER and FAO (2016) declared that "the training becomes the first step toward the self-help to take responsibilities on their destination, or what is known as self-management."

It is necessary to train them, for them to be able to make use of the forest resources, including the native fruits. These strategies of multiple uses would allow them to maintain a dual economy. On the one hand, they have production areas from which they sell some goods and services, and on the other hand, they satisfy their necessities of local consumption (García-Frapolli *et al*., 2008; Infante-Ramírez, 2011; Infante-Ramírez and Arce Ibarra, 2013).

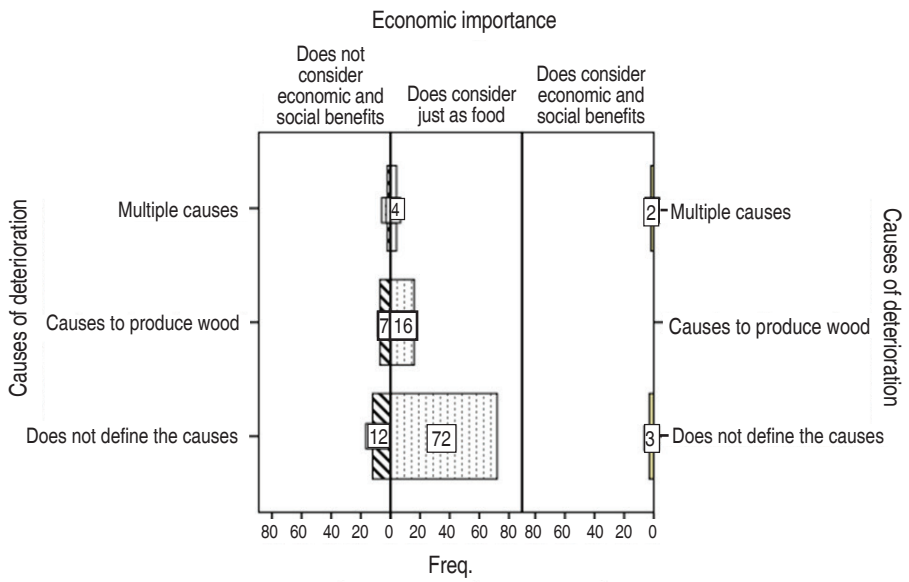

Figure 2. Distribution of frequencies according to the perception of the local actors about the causes of deterioration and economic importance.

Figure 3 shows the relationship between the proximity to the forest and the knowledge of the fruit species. In this case, the positions were favorable because all the local actors were in the advanced and intermediate positions, so they indeed know the native fruit species. It is observed that the 87% identify the 100% of the fruits (103/118) and that the largest representativeness of them (58) comes from those who live nearer the forest. The latter is an important question to be kept in mind according to the criteria of Mitjans (2012), due to the people know their region, the traditional use of the natural resources, the location of the species and in some cases the way of plants propagation. These factors are important inquiries to be considered in the management plan of the different forest ecosystems.

According to Moreno (2013), during the last decades some geographers, anthropologists, sociologists, historians, naturalistic, jurists, among other specialists have continued the task of describing and building theories about the collective rural institutions and their knowledge and perceptions about the natural resources.

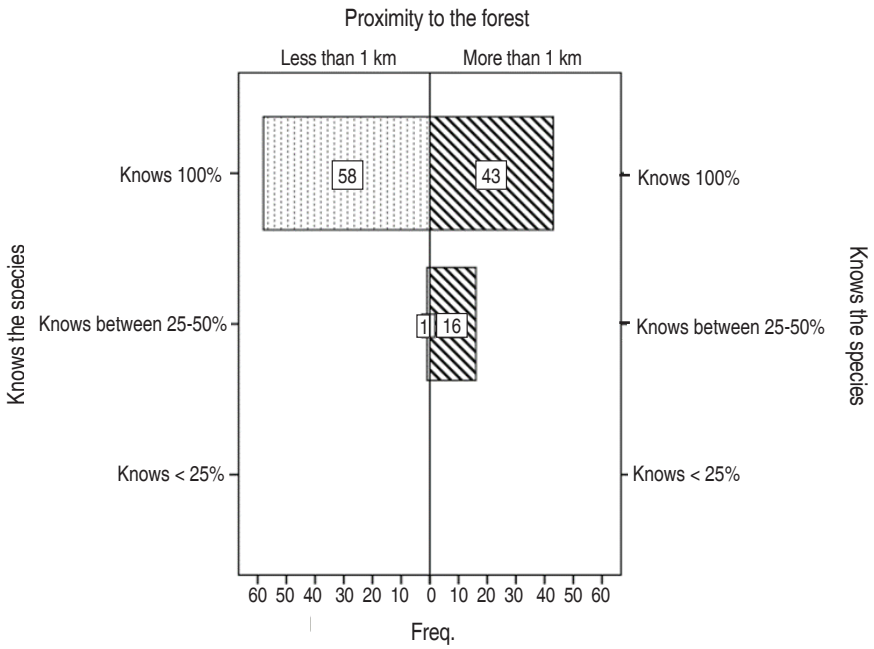

Figure 3. Distribution of frequencies according to the perception of the local actors on knowledge of the species and proximity to the forest.

Concerning the knowledge of the species in function of the school level of the local actors, it was verified that all of them know the native fruits since they were in the visions 2 and 3. 85% (100/118) of the interviewees know the 100% of native fruit species. Individuals in low positions were not found (Figure 4).

Traditional knowledge and the local actors participated in the administration of the natural resources should be considered during their management. Molina-Pelegrín *et al.* (2011) obtained important results concerning protected and conserved areas, with the intervention of the adjacent communities.

Likewise, Vargas Larreta (2013) asserted that it is necessary to use the local and traditional knowledge about the biodiversity to incorporate it in the forest planning and management to guarantee better results in their conservation.

On the other hand, Jiménez (2012) alluded to the relation between the anthropic action and distance from the forest, asserting that local players who are close to the forest know the species and their uses.

According to Packham (1993), although it exists a growing awareness of the importance of the wild fruits and other

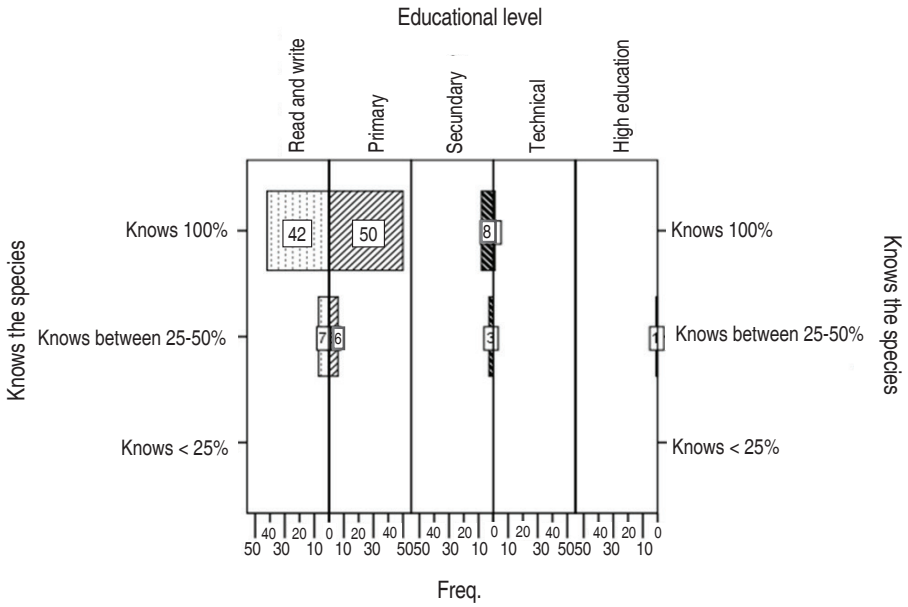

Figure 4. Distribution of frequencies according to the perception of the local actors on knowledge of the species and the school level.

non-mature stand products from the forests, it is still a very little knowledge of their importance to keeping the families safe during difficult climatic, nutrition, and financial periods. This short literature review seems to show that the social and economic security should be attributed to the highly diverse environment that sustains these forested areas of Miombo (Africa). The perception of the local actors on the environmental function was contrasted with the knowledge they had about the species, resulting in all local actors know the fruit species, while only the 46% recognized their environmental importance (Figure 5).

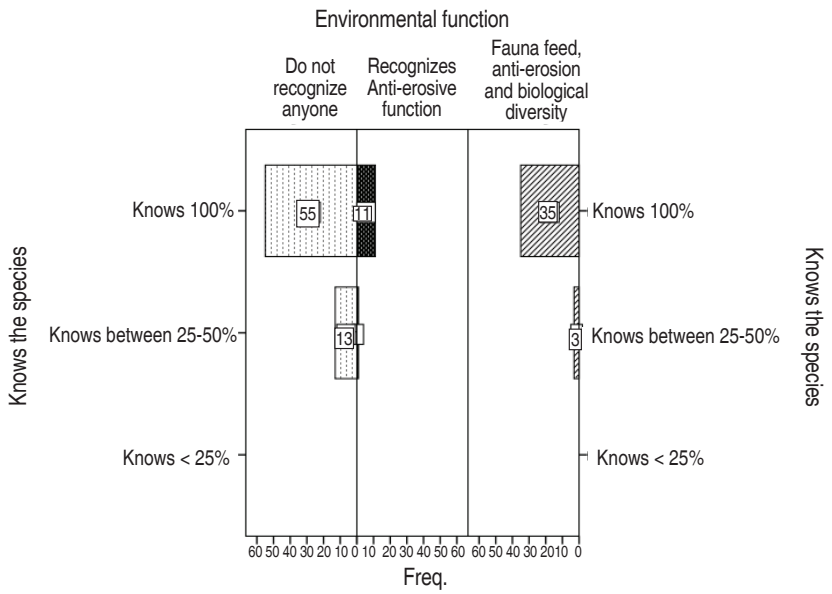

Figure 5. Distribution of frequencies according to the local actors' perception of the environmental function and the knowledge about the species.

The environmental perception is an important element to consider for the environmental administration since it reinforces the responsibility level and the local actors' right to the forest (Mitjans, 2012).

From the results of the correlation analysis (Table 2) by applying the coefficient of Rho of Spearman, it is observed a significant relationship between the variables causes of deterioration and economic importance. The relation among these variables is inverse, which indicates that the lower the economic importance, the increasing in the causes of deterioration. It is conditioned by the lack of knowledge about the economic, environmental, and nutrition importance of the native fruits. It is an issue of great importance for the African countries since different studies in other parts of this continent have concluded that the wild fruits provide an important dietary component, mainly to the children (Packham, 1993).

Table 2. Correlation analysis between the studied variables according to the correlation coefficient Rho of Spearman.

	Environmental Function	Economic Importance	Knowledge of the species	Deterioration Causes
Environmental Function	1.000	-0.102	-0.072	0.05
Economic Importance	-0.102	1.000	0.137	-0.192*
Knowledge of the species	-0.072	0.137	1.000	-0.01
Deterioration Causes	0.05	-0.192*	-0.01	1.000

*: $P<0.05$

Strengths and weaknesses found in the community of Munhiba

During the participatory diagnosis were also identified strengths and weaknesses that allowed to propose a group of working lines to facilitate the participatory NF self-management by the community of Munhiba.

Strengths

1. There is a diversity of NF species in the community.
2. Suitable edafo-climatic and ecological condition for NF production.
3. Conducted studies show that Mozambique's NF have potential uses (feeding, medicine, the industry of cosmetics, building supplies, wood, and extraction of essential oils).
4. Many of the NF are protected by beliefs and traditional myths.
5. Most of the local actors use the NF as the staple and daily food due to the economic situation and precarious conditions in which they inhabit.
6. There is an available budget for projects on feeding.
7. Simple technology and low-cost expenses will be required if local actors are included in the handling of NF.
8. A high percentage (82%) of the interviewees knew 100% of the NF of the study area.

Weaknesses

1. Limited community knowledge on NF nutritional security.
2. The absence of systematic and participatory approaches through which the community could negotiate its patrimony in an effective participatory way.
3. Insufficient availability of upgraded technical people in the community institutions to conduct NF research and production processes.
4. Inadequately qualified technicians in the necessary research areas.
5. Insufficient technical support for the producers.
6. Low applied investment in the research and implementation projects on NF.
7. There is not an intentioned production and processing of NF, so it responds to the necessities of the community.
8. The community does not value the NF importance to improve their economy and quality of life.
9. There is not an integral economic, environmental and social perception to facilitate the suitable NF self-management.
10. Faulty environmental management that leads to the NF exploitation from inadequate uses, as it is the felling for firewood and coal.

11. Insufficient training of the community on the participatory processes in NF administration and use.
12. Annual practices of forest fires in the herbaceous stratum where the NF are.

These elements related to the strengths and weaknesses show that it is necessary to promote community work. According to the approaches of Reyes *et al.* (2017), it becomes more and more opportune and indispensable for the economic and social development of a country.

Just as it is reflected in Table 3, there were identified 17 native fruits species, distributed in 14 families and 16 genders, which are used for feeding, the control of the illnesses, and sorcery; as the local actors identify in a territory. The family with more representative species is Malvaceae with three species (*Adansonia digitata* L., *Anzanza garckeana* F. Hoffm, and *Ceiba pentandra* L.).

Table 3. Identified species in the study area.

No.	Common name	Scientific name	Family
1	Malambe- imbondeiro	*Adansonia digitata* L.	Malvaceae
2	Maebe	*Annona senegalensis* Pers.	Annonaceae
3	Maceraou umtanwala	*Ancylobotrys petersiana* Klotzsch.	Apocynaceae
4	Mukole	*Anzanza garckeana* F. Hoffm.	Malvaceae
5	Sumauma	*Ceiba pentandra* L.	Malvaceae
6	Tongoma	*Eugenia mosambicensis Engels.*	Myrtaceae
7	Nalu	*Ficus sycomorus* L.	Moraceae
8	Tubi	*Parinari curatellifolia Planch.*	Chrysobalonaceae
9	Chindo	*Phoenix reclinata* L.	Palmaceae
10	Matema	*Strychnos madagascariensis* Lam.	Loganiaceae
11	Massala, mutamba	*Strychnos spinosa* Lam.	Loganiaceae
12	Missica,uepa	*Tamarindus indica* L.	Fabaceae
13	Mafureira	*Trichilia emetica* Vahl subsp.	Meliaceae
14	Nyunkomazhanje.	*Uapaca kirkiana* Müll. Arg.	Phyllanthaceae
15	Mangiro, maphilo,	*Vangueria infausta* Burch.	Rubiaceae
16	Puro	*Vitex doniana* Sweet.	Lamiaceae
17	Pudho	*Zanha africana* (Radlk) Exell.	Sapindaceae

It is important to underline that some of the NF species found in the area were already studied by Cândido (2011) in the district of Quissanga, such as the case of *Annona senegalensis*, *Ancylobotrys petersiana*, *Phoenix reclinata*, *Adansonia digitata*, *Ceiba pentandra*, *Strychnos madagascariensis*, *Tamarindus indica*, *Trichilia emetica*, *Anzanza garckeana*, *Vangueria infausta*, *Zanha africana*, and *Vitex doniana*. This author explained that they are widely used as food and commercialized by the local actors.

Table 4 shows different uses of the species by category. Among the species three of them (*Phoenix reclinate* L., *Tamarindus indica* L., and *Trichilia emetica* Vahl subsp) have a wide scope of uses.

Out of the 118 interviewees, 77 (65%) declared that they eat the leaves of the *Ceiba peltandra* and its toasted seeds.

Among the identified species, it was found the *Strychnous madagascariensis*, which is used as a food and medicine, has been yet studied by Inguane *et al.* (2015). They found in it a considerable percentage of sugars, lipids, proteins, macronutrients (K, Na, Mg, and Ca), micronutrients (Cu, Ni, Zn, Mn, and F), as well as a content of vitamin A, alkaloids, and saponins (anti-cholesterol and anti-cancer).

A high nutritional and medicinal potential can be exploited for nutrition security during scarcity periods.

Striycnos spinosa Lam. It is a fruit used for feeding, liquor production, and in the traditional medicine for illnesses treatment. Other authors like Munyemana *et al.* (2015) found similar results in conducted studies.

Among the interviewees, 79% (93/118) identified the NF as a necessary food and medicine source for their life development.

Table 4. Category by use declared of the species.

No	Scientific name	Category of use			
		Food	Medicine	Wood	Other uses
1	*Adansonia digitata* L.	x	x		
2	*Annona senegalensis* Pers.	x	x		
3	*Ancylobotrys petersiana* Klotzsch.	x			
4	*Anzanza garckeana* F. Hoffm.	x	x		x
5	*Ceiba pentandra* L.	x		x	x
6	*Eugenia mosambicensis* Engels.	x	x	x	
7	*Ficus sycomorus* L.	x	x		
8	*Parinari curatellifolia* Planch.	x	x	x	
9	*Phoenix reclinata* L.	x	x	x	x
10	*Strychnos madagascariensis* Lam.	x	x		
11	*Strychnos spinosa* Lam.	x	x	x	
12	*Tamarindus indica* L.	x	x	x	x
13	*Trichilia emetica* Vahl subsp.	x	x	x	x
14	*Uapaca kirkiana* Müll. Arg.	x	x		
15	*Vangueria infausta* Burch.	x	x		
16	*Vitex doniana* Sweet.	x		x	x
17	*Zanha africana* (Radlk) Exell.	x			

Working lines proposed for the NF self-management in the community of Munhiba

The inadequate NF management caused insufficient protection of them, leading to the damage of forest ecosystem, where they are. Besides, not taking advantage of their total benefit as nutritious potential and source of employments and earnings for the local actors in the community of Munhiba.

The proposed working lines aim to place the local actors as the immediate beneficiaries; they leaned on in their suggested ideas (from the groups' discussion) to help the promotion of a functionally participatory development process under a sociocultural vision. These work lines have been figured out as follows:

1. To carry out exchange participatory meetings with the community and the institutions, applying participatory technics to the creation of democratic knots that allow, in a combined way, the decision-making about the NF management.
2. Upgrading the population's knowledge, both individual and collective, to assume in a participatory way the administration of the NF in their territory.
3. To elaborate and bring into the forest management, practical plans with an integral and holistic vision of the forest ecosystem, with specificities in the NF; contributing with intellectual instruments and supported on methodological tools that allow accessing and building up an environmental knowledge.
4. Designing and implementing integrated programs, to maximize the use and exploit the potential of each species.
5. To identify the more outstanding NF at the economic level and to adopt measures for their great scale domestication and production.

6. Implementing programs of secondary and high education students' formation, as well as technical education level in different application areas to the NF.
7. To document the local knowledge on NF (ethnobotanical studies) and to create mechanisms of popularization of the local know-how.
8. Promoting in the community the nutritive, medicinal, and commercial importance that have the NF.
9. To train the local actors in how to manage the NF.
10. To make a catalog that provides information regarding NF nutrition security and the collateral benefits for their management in a participative way.
11. To carry out protection practices against fires, pests, diseases, and anthropogenic activities.

CONCLUSIONS

The developed diagnosis and its analysis facilitated to verify the limited participation and local actors' knowledge (producers, processors, and merchants of fruits) from Munhiba town. The lack of work lines was also verified for a community self-management, so that contributes to its production and commercialization.

Despite the degradation by the man's influence in the study area, it is evident the high potential of NF mean for the community, mainly as edible, medicinal, and wood use, represented by 17-identified species; some of them of high commercial value as *Vangueria infausta* (Mangiro), *Strychnos spinosa* (Massala) and *Trichilia emetica* (Mafurra).

The working lines for NF self-management proposals in the community of Munhiba are based in the developed diagnosis and the theoretical conceptions, and they are conceived to be executed with a participatory social focus that contributes to their appropriate management.

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Incidence of operative parameters in the production of biohydrogen generated from urban organic waste

Incidencia de parámetros operativos en la producción de biohidrógeno generado a partir de residuos orgánicos urbanos

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Keywords:
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ABSTRACT

Organic waste is considered a substrate of great interest to produce biohydrogen. In the present work, the influence of some physical and chemical parameters in the operation of a bioreactor for biohydrogen generation were studied, taking as a substrate organic residue from a wholesale food market without adding inoculum. Therefore, an experimental design of central composition was made, with four factors and two levels. The dependent variables were maximum hydrogen content (% of H_2), daily production of hydrogen (L H_2 d^{-1}) and its cumulative production (L H_2). The independent variables were operation pH (pH_o), pH of acidification (pH_a), the duration time of the acidification stage, and stirring. A numerical optimization was carried out, allowing the prioritization of the factors, and maximizing the response variables. Resulting in a yield of up to 14.9 L H_2 d^{-1}, a hydrogen content of 49.2% and a cumulative production of 21.6 L H_2, for pH_a values of 4.9; pH_o between 6 and 6.1; acidification time of 2 d and stirring of 41.4 rpm. Likewise, a graphical optimization was carried out, reaching 14.9 L H_2 d^{-1}, a hydrogen content of 44.2% and an accumulated 22.8 L H_2, for pH_a values between 4.5 and 4.95; pH_o between 5.6 and 6.3; acidification time of 2 d, and stirring of 37.1 rpm. Maximum yields were 1.9 L H_2 $L_{waste.day}^{-1}$, 4800 mL H_2 g_{COD}^{-1}, and 608.6 m L H_2 $g_{TVSadded}^{-1}$, values similar to those reported by other authors using organic waste in the production of hydrogen, using inoculum.

Palabras clave:
Etapa de acidificación
Fermentación
Optimización
Materia orgánica
Energía renovable
Agitación

RESUMEN

Los residuos orgánicos son considerados sustratos de gran interés para la producción de biohidrógeno. En el presente trabajo se estudió la influencia de algunos parámetros físicos y químicos en la operación de un bioreactor para la generación de biohidrógeno, tomando como sustrato residuos orgánicos provenientes de una central de abasto sin adicionar inóculo. Para ello se realizó un diseño experimental de composición central, con cuatro factores y dos niveles. Las variables dependientes fueron el contenido máximo de hidrógeno (% de H_2), la producción diaria de hidrógeno (L H_2 d^{-1}) y su producción acumulada (L H_2). Las variables independientes fueron, pH de operación (pH_o), pH de acidificación (pH_a), tiempo de duración de la etapa de acidificación y agitación. Se realizó una optimización numérica que permitió priorizar los factores y maximizar las variables de respuesta, obteniéndose hasta 14,9 L H_2 d^{-1}, contenido de hidrógeno de 49,2% y una producción acumulada de 21,6 L H_2, para valores de pH_a de 4,9; pH_o entre 6 y 6,1; tiempo de acidificación de 2 d y agitación de 41,4 rpm. De igual forma se realizó una optimización gráfica alcanzándose 14,9 L H_2 d^{-1}, un contenido de hidrógeno de 44,2% y 22,8 L H_2 acumulado, para valores de pH_a entre 4,5 y 4,95; pH_o entre 5,6 y 6,3; tiempo de acidificación de 2 d y agitación de 37,1 rpm. Los rendimientos máximos fueron de 1,9 L H_2 $L_{residuo.dia}^{-1}$, 4800 mL H_2 g_{DQO}^{-1} y 608,6 mL H_2 $g_{SVadicionado}^{-1}$, valores similares a los reportados por otros autores empleando residuos orgánicos en la producción de hidrógeno usando inóculo.

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Urban organic solid wastes are generated by the communities that inhabit the so-called urban centers. This urban organic waste, also called waste biomass, is comprised of considerable amounts of peels, fruits, and vegetables in an advanced decomposition process (UPME, 2009). Organic waste consists mainly of carbohydrates, starch, protein, small amounts of cellulose and hemicellulose (Park *et al*., 2010). It represents a source of bioenergy with the potential to reduce the current environmental and energy problems, contributing to the reduction of greenhouse gases, and the acquisition of CO_2 emission rights (Robledo-Narváez *et al*., 2013). The transformation of organic waste by anaerobic fermentation allows better use of it, generating a renewable gas that helps to reduce the consumption of fossil fuels, and the emission of greenhouse gases; especially, if during the process, the metabolic path is oriented to the production of hydrogen instead of methane.

Hydrogen has a calorific value of 122 kJ g^{-1}, which is 2.75 times higher than fossil fuels. This feature has made it a promising alternative fuel; especially, considering that its combustion does not generate polluting emissions. It can also be used for the generation of electric power using it directly in internal combustion engines, in thermal turbine systems or in fuel cells (Kim *et al.,* 2009). In recent years, the production of hydrogen by fermentation, has aroused considerable interest because of the diversity and relatively low cost of the substrate (Lin J *et al*., 2011) since the yields that can be obtained (3.0 moles of H_2 mol glucose^{-1}) and the content of hydrogen in the gas, up to 63% (Papadias *et al*., 2009).

Despite the comparative advantages of organic waste in the production of hydrogen by fermentation, its use has rarely been reported. Hernández *et al.* (2014) used coffee mucilage in co-digestion with pig manure to generate hydrogen, obtaining a maximum production rate of 7.6 NL H_2 $L_{of\ mucilage.day}^{-1}$, and hydrogen content in the gas up to 39%. Mohan *et al*. (2009) showed the viability of hydrogen production from plant residues, indicating that its generation depends on the concentration of the substrate and its composition. Gómez *et al*. (2009) studied the behavior of organic waste with an inoculum obtained from a municipal wastewater treatment plant, indicating that a low organic loading speed favors the fermentation performance. The authors report that the maximum production of hydrogen was 67 L H_2 $kg_{TVSadded}^{-1}$, and although the yield was unstable, its recovery could be achieved by stirring the mixture, suspending the feed, and controlling the pH in the range of 5-5.5.

Gómez-Romero *et al*. (2014) studied the co-digestion process of raw cheese whey with fruit vegetable residues for the production of biohydrogen, using five C/N ratios (7, 17, 21, 31, and 46) at a pH of 5.5 and 37 °C. The highest yield was 449.84 mL H_2 g_{COD}^{-1} for a C/N ratio of 21. The reported pH range for maximum hydrogen production is between 5.0 and 6.0. Wang and Wan (2008) report an optimum pH around 5.5 when anaerobic sludge, sucrose or glucose is used as a substrate in batch and continuous cultures. However, given the complex composition of organic waste, it is necessary to study the effect of pH on the hydrogen production when said substrates are used. The pH of the medium affects the yields of the hydrogen production, the type of organic acids produced, and therefore the specific speed of hydrogen production (Wang and Wan, 2008).

Although the pH impacts the hydrogen production by fermentation, its generation depends on multiple variables such as the type of substrate, temperature, organic loading speed, inoculum, type of reactor, among others. Authors such as Lin CY *et al*. (2011) and Wang and Wan (2008) carried out studies on the optimization of fermentation processes, finding that the experimental method based on response surface methodology (RSM) allowed them to represent the interaction between variables, to minimize the error in determining the effect of the parameters, and to determine the optimal conditions of operation. Therefore, it is considered an appropriate technique to optimize the fermentative hydrogen production (Muñoz-Páez *et al*., 2012).

The evolution of hydrogen by fermentation has also been studied through kinetic models such as Monod and Gompertz, the latter being the most used to describe the progress of microbial growth, substrate degradation, soluble metabolites production, and hydrogen production in batch fermentation (Chang *et al*., 2011). This equation has been used with great fit (R^2>0.90) by different authors such as Luo *et al*. (2011), who wanted to correlate experimental

results with a mathematical model. The Gompertz model is an empirical expression of three parameters that are experimentally adjusted: lag phase time (λ), potential H_2 production (H_{max}), and hydrogen production rate (R_{max}). Despite the fact that with it, high correlation coefficients between the observed and adjusted data from the hydrogen production are obtained (Wang and Wan, 2008), the three parameters of the model are limited to the specific experimental conditions, that is to say, the experimental conditions of each research.

The present study used an experimental design, based on the response surface methodology (RSM), to carry out a planned number of experiments and analyze the responses statistically, identifying the individual and interactive effects of the pH of acidification, the operation pH, the acidification time, and the rate of stirring, in relation to the production of hydrogen using urban organic waste as a substrate. The aim of this work was to analyze the incidence of some physical and chemical parameters in the hydrogen production when urban organic waste is used as a substrate, in which is believed that the pH of acidification and the acidification time have more incidence in the hydrogen production than the operation pH and stirring speed.

MATERIALS AND METHODS

A set of tests were structured based on an experimental design of central composition with four factors and two levels in each factor. The levels of the factors were taken from results achieved in preliminary tests (data not shown). The substrate used corresponded to a mixture of organic waste of fruits and vegetables not suitable for consumption due to mechanical damage and phytosanitary problems, coming from the Central Mayorista de Antioquia (CMA). These were reduced in size and feed to a reactor in a 1:1 ratio between water and substrate. The same waste composition was used in each test. The physicochemical analysis that was performed on the substrate included the determination of the Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Soluble Solids (TSS), Total Volatile Solids (TVS), and Volatile Fatty Acids (VFA). For this procedure, two samples of 500 mL were taken at the beginning and the end of each fermentation. The concentrations of BOD (5210-B), COD (5220-B), VFA, TSS (2540-B) and TVS (2540-E), were carried out according to the analytical methods of the Standard Methods for the Examination of Water and Wastewater of the APHA-AWWA-WEF, 19th edition of 1995.

The independent variables or factors were the acidification time (t_a), operation pH (pH_o), pH of acidification (pH_a) and stirring (w). The response variables were hydrogen production (HP, L H_2 d^{-1}), cumulative hydrogen production (CHP, L H_2) and maximum content of hydrogen in the gas (MCH, $\%H_2$). To determine the response variables a gas meter (Metrex G2.5 with precision of 0.040 m^3 h^{-1} and a maximum pressure of 40 kPa) with a silica gel humidity trap was coupled. Of the gas produced per day, a sample was taken in Tedlar bags with a capacity of 1 L, using 85% of its volume to be evaluated by means of gas chromatography. For this, a gas chromatograph (GC, Varian 3800) equipped with a thermal conductivity detector (TCD) was used, with columns connected in series Hs N6-07N (Hayesep), and Ms 13x4-09N (Molesieve) with a BackFlush-Bypass system, oven temperatures and detector of 40 and 170 °C respectively.

Regarding the independent variables, the pH was monitored daily with a portable pH-meter HI 98103 Hanna Instruments, equipped with a standard LB electrode with a range of 0-14 pH, resolution of 0.01 pH and accuracy of ±0.2 pH. For stirring, was used a helical ribbon impeller, coupled to a gear-motor, a speed variator and a timer. Three stirring rates were defined according to preliminary tests; all of them were applied during five minutes with a frequency of every hour. The acidification time (t_a) consisted in the number of days elapsed from the beginning of the fermentation until the addition of a base (agricultural lime) was started.

The results in each response variables were fitted to multivariable second-order polynomial models, where the "backward" method was used to select the significant parameters in each model. The verification of the fit of the models was made using variance analysis with a significance level of 5%. Besides, an optimization was carried out in order to maximize the response variables, to obtain the combination of these responses and establish under these conditions the values of the factors. The fit and optimization of the models was done through the Design Expert V9® software. The optimization process followed the method of statistical desirability, in addition a canonical correlation analysis of the response surfaces in the variables was carried out, and the stationary point was located in the experimental region with the SAS software, version 9.0®.

Besides to the model obtained by non-linear regression, the modified Gompertz model was used to describe the Cumulative Hydrogen Production (equation 1).

$$H = P_{\exp} * \left\{ -\exp\left[\frac{R_{\max} * e}{P} * (\lambda - t) + 1 \right] \right\} \quad (1)$$

Where: H is the cumulative production of hydrogen (mL), λ the lag phase time (h), P is the potential production of hydrogen (mL), R_{max} is the maximum rate of hydrogen production (mL h^{-1}) in the time interval, t corresponds to the hours per day of biohydrogen production and e is 2.718281828 (Valdez-Vazquez and Poggi-Varaldo, 2009). The cumulative production of hydrogen (H) was obtained by adding the liters registered in each test every day, from the first day until the presence of hydrogen in the gas ceased. The lag phase time corresponded to the days elapsed from the moment the lime was added to the bioreactor until the production of hydrogen began. The potential production of hydrogen (P) was the cumulative total of the production in each test, while the maximum production rate was given by the ratio between the maximum value of hydrogen production and the hours that were required for it in each test. The fit of the experimental data to the Gompertz model (R^2 and R^2_{adj}) was done with the Curve Fitting Tool (CFTools) from Matlab, version 2012®.

All experimental tests were carried out in a stainless-steel bioreactor, hermetically sealed, with a volume capacity of 20 L and operated at 30 °C in batch mode. The tests were performed in the Agricultural Mechanization Laboratory of the Faculty of Agricultural Sciences of the Universidad Nacional de Colombia, Medellín campus.

RESULTS AND DISCUSSION

Characterization of the substrate

The substrate was comprised of a mixture of green leaves waste (cabbage and lettuce) and fruits (papaya, mango, guava, and orange). The results of the initial and final physicochemical characterization of the substrate are shown in Table 1. Tests E1, E2 and E5 showed inconsistent results, attributed maybe to the lack of uniformity in the size of the waste at the time of the analysis (data not shown).

Table 1. Physicochemical characterization of the raw material.

Test	VFA (meq L_{VFA}^{-1})	COD (mg O_2 L^{-1})	TVS (mg L^{-1})	TS (mg L^{-1})	BOD (mg O_2 L^{-1})
E3- I	490	58,500	50,200	79,900	32,050
E3-F	470	53,500	22,280	64,780	23,032
E4- I	660	60,000	16,020	33,040	20,433
E4-F	450	50,000	15,460	55,600	25,183
E6-I	7500	54,250	38,760	58,640	34,833
E6-F	6550	37,500	20,560	55,540	25,608
E7-I	3300	55,750	35,720	68,520	39,333
E7-F	2850	42,500	29,240	51,900	25,650
E8-I	3660	47,500	25,900	42,180	23,733
E8-F	2860	43,750	16,280	45,720	24,967
E9-I	3620	75,750	49,120	66,040	38,417
E9-F	2420	70,750	28,480	80,280	30,250
E10-I	3440	72,500	41,460	63,080	36,167
E10-F	3440	55,000	36,440	89,560	22,475
E11-I	4380	50,000	24,460	38,520	20,308
E11-F	3400	45,000	16,860	50,040	23,000
E12-I	3600	67,500	61,740	79,200	33,583
E12-F	1680	66,750	42,020	88,120	26,583
E13-I	5160	100,750	50,900	68,160	32,000
E13-F	2360	76,750	36,420	78,800	29,250
E14-I	3940	80,750	58,520	77,100	36,583

Continuation Table 1

Test	VFA (meq L_{VFA}^{-1})	COD (mg O_2 L^{-1})	TVS (mg L^{-1})	TS (mg L^{-1})	BOD (mg O_2 L^{-1})
E14-F	3860	75,000	32,160	76,460	30,000
E15-I	3640	63,250	45,180	64,860	24,725
E15-F	3440	43,250	43,580	58,520	22,458
E16-I	3820	86,750	35,440	91,160	34,417
E16-F	3560	70,000	31,900	71,440	26,750
E17-I	3920	62,875	21,300	50,920	20,833
E17-F	3840	53,375	19,200	25,880	16,792
E18-I	3400	77,125	36,740	83,880	37,750
E18-F	3180	64,625	27,600	56,800	18,458
E19-I	3920	52,125	30,360	69,400	35,208
E19-F	3480	48,375	22,700	60,960	23,250
Average	3322	61,391	33,219	63,906	27,940

E: Test **I:** Initial **F:** Final

The percentages of organic matter removal varied between 1 and 36%; the highest COD value 100,750 mg O_2 L^{-1} was observed in test E13, in which a 24% of organic matter was removed, and a 15.2% of hydrogen in the gas was obtained. An increase in the percentage of removal, up to 18%, was observed at high concentrations of organic matter. This increase may happen because with the increment in the organic load there is a greater amount of carbohydrates and hemicellulose available to be used as a substrate by the bacterial population (Mohan *et al*., 2009). However, the maximum production did not occur with the highest COD value. This discrepancy can be explained since high initial values of COD can generate an accumulation of metabolites and instability in the pH (Redondas *et al*., 2012).

It has been reported that the adequate concentration of Total Solids (TS) to obtain hydrogen from organic waste varies between 1.3 and 50 g L^{-1} (Sekoai and Gueguim Kana, 2013). In the present work, the concentration of TS ranged from 25.8 to 91.2 g L^{-1}. For this last value, the content of H_2 in the gas decreased, while the CO_2 increased to more than 70%, similar results to those presented by Rangel (2011). Likewise, the hydrogen content in the gas was low or zero for tests E7, E15, and E18, in which there was a concentration of total solids of 68.5, 64.8, and 83.8 g L^{-1} respectively. This situation coincided with the report of others authors, Liu *et al*. (2009) studied the production of hydrogen from organic solid waste at different TS concentrations.

Volatile Fatty Acids (VFAs) showed a wide range of variation, from 350 to 7500 meq L_{VFA}^{-1}, this last value was the initial value for test six in which a maximum hydrogen percentage of 6.41 was obtained. High values of VFA, higher than 3000 mg L^{-1} (3000 meq L_{VFA}^{-1}), generate a VFA accumulation that favors the depletion of the buffer capacity of the substrate, affecting directly the pH, which plays a crucial role in the hydrogen production and in the growth of the acidogenic microbial population (Elbeshbishy *et al*., 2011).

Hydrogen production and performance indicators

The hydrogen production (HP) ranged between 0 and 14.4 L d^{-1}, the maximum hydrogen content in the gas (MCH) was between 0 and 42.4%, and the cumulative production of hydrogen (CPH) was between 0 and 20.4 L (Table 2). In some tests, there was not hydrogen production, as was the case of tests 1, 15, 18 and 19. The highest values of hydrogen production were observed in tests 2, 4 and 8 with 14.4, 9.8 and 6.4 L H_2 d^{-1} and a hydrogen content in the gas of 36.9, 42.4 and 24.5%, respectively. The mentioned tests had acidification times of 2.2 and 1 day. The hydrogen production per day was higher compared to those obtained by other authors such as Ueno *et al.* (2007) and Kim *et al.*

(2008), who in tests with organic and restaurant waste found productions of 5.4, and 1.47 L H_2 d^{-1}. Concerning hydrogen content, the values obtained were lower than those reported by these same authors (55%), with a maximum value of 42.4%.

The maximum daily and cumulative production were obtained for pH_a, pH_o, and stirring of 4.55, 5.93, and 37.8 rpm, respectively. In turn, the highest hydrogen content in the gas was reached for pH_a, pH_o, and stirring of 4.65, 5.84, and 37.8 rpm, respectively. These results coincide with those given by other authors such as Fernández *et al.* (2010), who using food waste under these conditions and with mesophilic temperature obtained high hydrogen production. Other authors (Valdez-Vazquez and Poggi-Varaldo, 2009) achieved up to 58% of hydrogen in the gas for a mesophilic regime, using an inoculum from wastewater.

Table 2. Production and test performance indicators.

Test	pH_a	pH_o	t_a (d)	w (rpm)	HP (L H_2 d^{-1})	MCH (%H_2)	CPH (L H_2)	Y_S (L H_2 $L_{waste.day}^{-1}$)	Y_{COD} (mL H_2 $g_{CODremoved}^{-1}$)	Y_{TVS} (mL H_2 $g_{VSadded}^{-1}$)
1	3.97	6.11	1	29.9	0.0	0.0	0.0	0.0	NA	NA
2	4.55	5.93	2	37.8	14.4	36.9	20.4	1.9	NA	NA
3	4.66	6.38	3	45.1	2.8	28.2	5.4	0.4	564	56.2
4	4.65	5.84	2	37.8	9.8	42.4	16.0	1.3	975	608.6
5	4.15	5.22	1	29.9	2.1	23.3	4.1	0.3	NA	NA
6	4.9	5.49	3	29.9	3.1	9.4	3.2	0.4	183.9	79.5
7	4.07	6.29	3	45.1	0.2	3.1	0.2	0.1	14.3	5.3
8	4.03	5.35	1	45.1	6.4	24.5	9.3	0.9	1701.3	246.3
9	4.94	5.51	1	45.1	0.7	4.6	0.9	0.1	140	14.3
10	4.9	5.42	1	29.9	2.4	18.5	3.9	0.3	137.7	58.1
11	4.16	5.56	3	45.1	2.2	26.9	3.2	0.3	430	87.9
12	4.61	5.98	1	45.1	3.6	10.9	6.9	0.5	4800	58.3
13	4.75	6.39	1	29.9	3.8	15.2	4.5	0.5	158.3	74.7
14	3.8	5.98	3	29.9	0.08	3.9	0.1	0.0	13.6	1.3
15	4.32	6.43	3	29.9	0	0.0	0.0	0.0	0.0	0.0
16	4.3	6.46	3	29.9	0.5	6.4	0.7	0.8	29.9	14.1
17	4.78	6.14	3	45.1	0.4	7.3	0.6	0.1	42.1	18.8
18	4.51	6.4	1	45.1	0	0.0	0.0	0.0	0.0	0.0
19	3.8	4.41	2	37.8	0	0.0	0.0	0.0	0.0	0.0

Ys: substrate yield, Y_{COD}: yield according to organic load, Y_{TVS}: yield according to total volatile solids, NA: Not Available.

The maximum yield of the substrate was of 1.9 L H_2 $L_{waste.day}^{-1}$ in test two. This result was obtained without using any inoculum. Shin *et al.* (2004) found yields of 0.33 L H_2 $L_{waste.day}^{-1}$ with waste from marketplaces in a continuously agitated bioreactor and an inoculum pretreated with high temperatures at intervals of 15 min for 2 d, and a pH of 6.5; reaching a hydrogen content in the gas of 13%. Robledo-Narváez *et al.* (2013), reported very similar values using a batch-type reactor with urban organic waste with inoculum and pH value of 6.8, the yield obtained was 0.27 L H_2 $L_{waste.day}^{-1}$.

The maximum yield reached, according to the organic load, was 4800 mL H_2 $g_{CODremoved}^{-1}$. This yield was higher than reported by other authors (Kim *et al.*, 2008) who obtained a yield of 128 mL H_2 $g_{CODremoved}^{-1}$, with organic

waste in batch fermentation and pH between five and eight. Other authors, instead, report that in fermentations oriented to the production of hydrogen using urban waste and sludge from treatment plants, the yield was 9873 mL H_2 $g_{CODremoved}^{-1}$, with pH between 5.5 and 6.0, and using inoculum pretreated with high temperatures (Zhu *et al*., 2008).

The maximum yield with respect to the volatile solids added (Y_{TVS}), was obtained in test 4 with 608.6 mL H_2 $g_{VSadded}^{-1}$, which corresponds to the test with the highest hydrogen content in the gas. A value much higher than reported by Nagao *et al*. (2012), who obtained yields of 48 mL H_2 $g_{VSadded}^{-1}$ with a mixture of urban organic waste in an operation pH range between 5.2 and 5.5, with operating conditions similar to those evaluated in this work. Lee *et al*. (2010) obtained yield of 118 mL H_2 $g_{VSadded}^{-1}$ with retention time of 96 d, for organic and restaurant waste. Other authors with crop and livestock waste have found that the yields can show significant variation, from 3 to more than 290 mL H_2 $g_{VSadded}^{-1}$, because of the different composition of the raw material (Guo *et al*., 2010).

Cumulative Production of Hydrogen (CPH) according to the modified Gompertz model

The CPH, according to the modified Gompertz logistic model, showed that the highest volume of hydrogen was reached in test 2 with 20.3 L H_2, followed by test 4 with 15.9 L H_2 (Figure 1). The production does not begin for all the tests at the same time because this depends on the acidification time used in each of them.

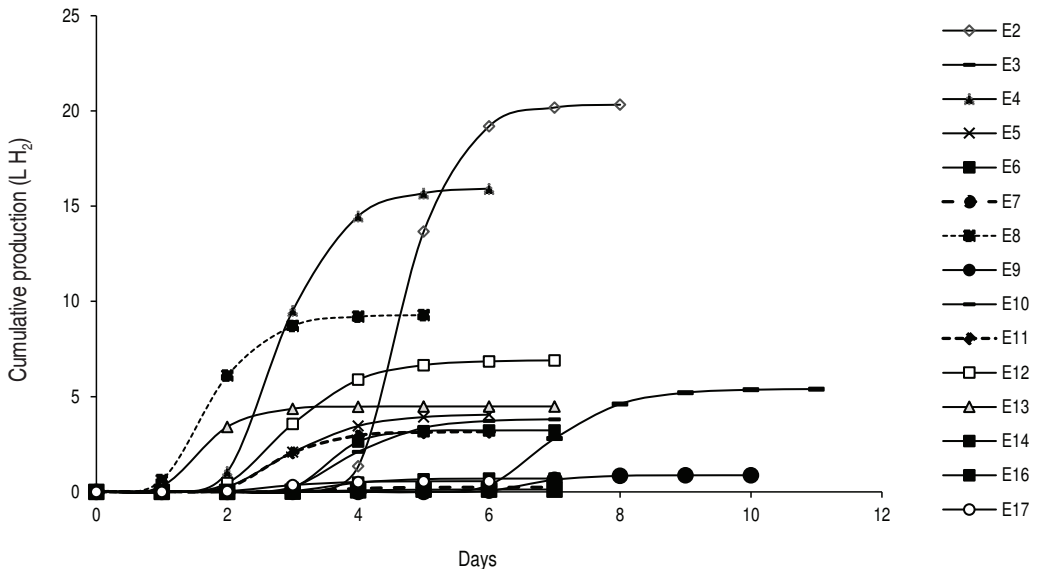

Figure 1. Cumulative production of hydrogen fitted to the modified Gompertz model.

The CPH increased until reaching its asymptotic value H_{max}. At this point, the daily hydrogen production ceased due to the depletion of the substrate given that the fermentations were made in batch. The experimental data were fitted appropriately to the modified Gompertz logistic model, obtaining multiple correlation coefficient higher than 0.99 (Table 3).

The highest hydrogen production speeds were observed in tests 2 and 4, at 599.6 and 406.2 mL H_2 h^{-1}, and adaptation times of 96 and 48 h respectively. Sharma and Li (2009) obtained hydrogen production speeds of 13 mL H_2 h^{-1} with urban organic waste and wastewater, with correlation coefficients higher than 0.95. Gadhe *et al*. (2014) found a delay in the hydrogen production speed with loads higher than 50 g_{COD} L^{-1}, coinciding with the results obtained in tests 3, 7, 9, 11, 14, 16 and 17 of this work, whose concentrations were between 50 and 86.75 g_{COD} L^{-1}. The results seem to indicate that with organic matter concentrations greater than 70 g_{COD} L^{-1} and acidification time of 3 d, there is a decrease in the average speed of hydrogen generation.

Table 3. Parameters of the modified Gompertz logistic model.

Test*	H_{max} (mL H_2)	R_{max} (mL H_2 h^{-1})	λ (h)	R^2
2	20,351.4	599.6	96	0.999
3	5409	117.5	144	0.999
4	15,975.7	406.2	48	0.999
5	4095	87.2	48	0.999
6	3227.6	128.7	72	0.999
7	234.6	7.8	72	0.999
8	9294.4	265.9	24	0.999
9	870.3	28.7	144	0.999
10	3834.8	88.8	72	0.999
11	3158.4	89.7	48	0.999
12	6917	149.9	48	0.999
13	4483.4	158	24	0.999
14	121.2	3.2	72	0.999
16	697.3	21.4	72	0.999
17	559.2	15.3	48	0.999

*Test 1, 15, 18 and 19 are not included since there was no hydrogen generation.

Statistical analysis and mathematical models obtained by regression

The analysis of variance for the different response variables according to a second-order polynomial quadratic model shows that in each variable the models are statistically significant (*P* <0.05, Table 4). Also, in none of the cases, there is influence of the individual effects. However, two of the quadratic effects (B^2 and C^2, operation pH and the acidification time, respectively) and combinations (AB and AD), have a statistically significant effect in the hydrogen production. The interaction between BC is significant only for the maximum content of hydrogen (MCH, %), and the cumulative production of said gas (CPH, L H_2).

Table 4. Variance analysis for quadratic polynomial models in the response variables (α=0.05).

Source	*P* value		
	HP (L H_2 d^{-1})	MCH (%)	CPH (L H_2)
Model	0.0010 *	0.0144 *	0.0004 *
A - pH of acidification	0.4968 NS	0.3526 NS	0.4643 NS
B - operation pH	0.5018 NS	0.1065 NS	0.4505 NS
C - Acidification time (d)	0.2153 NS	0.9587 NS	0.1111 NS
D - stirring (rpm)	0.7327 NS	0.6990 NS	0.8790 NS
AB	0.0261 *	0.0108 *	0.0121 *
AD	0.0147 *	0.0535 *	0.0159 *
BC	0.1055 NS	0.0476 *	0.0304 *
B^2	0.0007 *	0.0010 *	0.0002 *
C^2	<0.0001 *	0.0113 *	<0.0001 *

NS: nonsignificant effect and *: significant effect.

In the regression models, the variation around the mean explained by them was higher than 83% (Table 5). This situation indicates that models adequately represent the experimentation and it can be used for predictive purposes in the variables evaluated. However, in the variable maximum content of hydrogen (MCH), the variation explained by the models, taking into account the number of terms, decreased to 65% (R^2 adjusted) which means the model could be reduced by eliminating the components that do not have a significant effect.

Table 5. Coefficients for polynomial quadratic models and adjustment.

Regression coefficient	HP (L H_2 d^{-1})	MCH (%H_2)	CPH (L H_2)
β_0	-154.135	-189.461	-202.521
βx_1	-38.658	-265.397	-65.581
βx_2	65.210	231.116	97.447
βx_3	21.472	5.780	28.139
βx_4	1.555	5.514	2.055
$\beta x_1 x_2$	9.816	57.218	15.440
$\beta x_1 x_4$	-0.360	-1.284	-0.474
$\beta x_2 x_3$	1.892	11.655	3.603
βx_2^2	-9.598	-43.734	-14.763
βx_3^2	-8.290	-18.490	-12.579
R^2	0.91	0.83	0.93
$R^2_{adjusted}$	0.82	0.65	0.86

x_1, x_2, x_3 and x_4 correspond to the variables pH of acidification, operation pH, acidification time and stirring respectively.

Numerical optimization of the models

The optimization allowed to maximize the hydrogen generation, estimating the maximum daily production at 14.9 L H_2 d^{-1} for pH_a values of 4.9, pH_o of 6.0, t_a of 2 d and stirring of 40.2 rpm. The maximum hydrogen content in the gas is estimated at 49.2% at a pH_a of 4.9, pH_o of 6.2; t_a of 1.9 d and stirring of 41.4 rpm. Likewise, the maximum cumulative production of hydrogen was of 21.6 L H_2, for a pH_a of 4.9, a pH_o of 6.08, a t_a of 2 d and stirring of 41.4 rpm. Values in the independent variables close to those mentioned above, also allowed to achieve the best results in hydrogen production during the experimentation. The simultaneous optimization of the three response variables showed that the fermentation should be carried out at a pH_a of 4.9, a pH_o of 6.0, a t_a of 1.9 d and stirring of 29.9 rpm. With these values, the daily production is estimated at 14.7 L H_2 d^{-1}, the maximum content of hydrogen in the gas in 50.1% and the cumulative production in 21.6 L H_2. In general, when the pH_a increases and the pH_o drops, or when the pH_a drops, and the pH_o increases, the production of hydrogen decreases taking as reference a two-day acidification time (Figure 2).

Graphical optimization

The graphical optimization displays the area of feasible response values in the factor space. Figure 3 shows the superposition of the contour plots of each variable, which allowed to find the intersection area that provided the best values for the multiple responses. The regions that did not meet the optimization criteria are shaded in dark gray, and in light gray the optimization area. The above-mentioned area was found for pH_a values between 4.5 and 4.95, pH_o between 5.6 and 6.3, acidification time of 2 d and stirring of 37.1 rpm; reaching a production of 14.9 L H_2 d^{-1}, a maximum content of hydrogen of 44.2% and a cumulative production of hydrogen of 22.8 L H_2.

Canonical analysis

With the canonical analysis, the second-order models were rewritten in their canonical form, that is to say, in terms of the canonical variables that are transformations of the coded variables obtained in the models. In addition, the response surfaces were characterized, finding for each model the coordinates of the stationary points, the type of point, and the surface orientation.

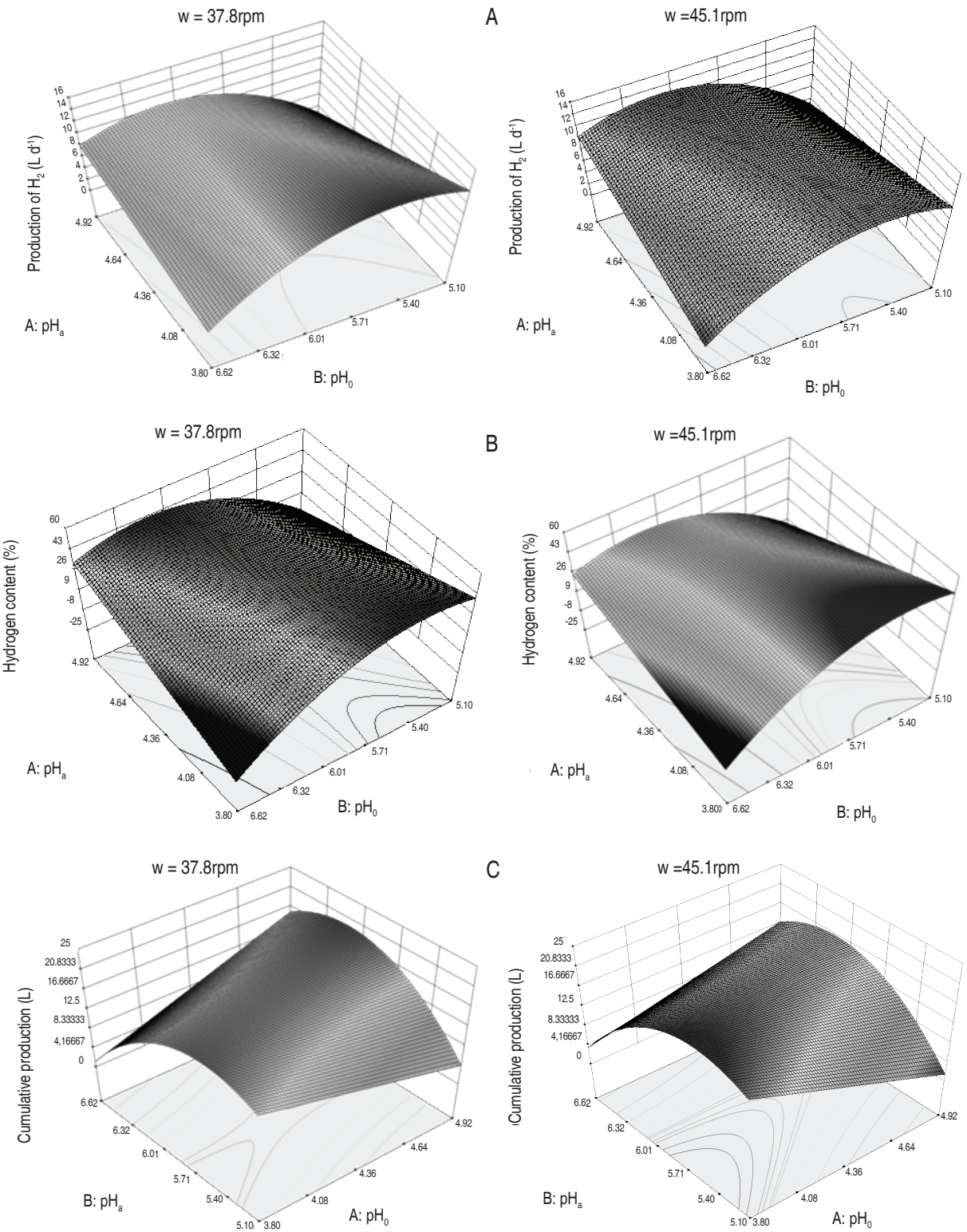

Figure 2. Response surface for the dependent variables. A. Hydrogen Production (HP); B. Maximum Content of Hydrogen (MCH); C. Cumulative Production of Hydrogen (CPH), for an acidification time (t_a) of two days and two stirring speeds, w=37.8rpm, w=45.1rpm.

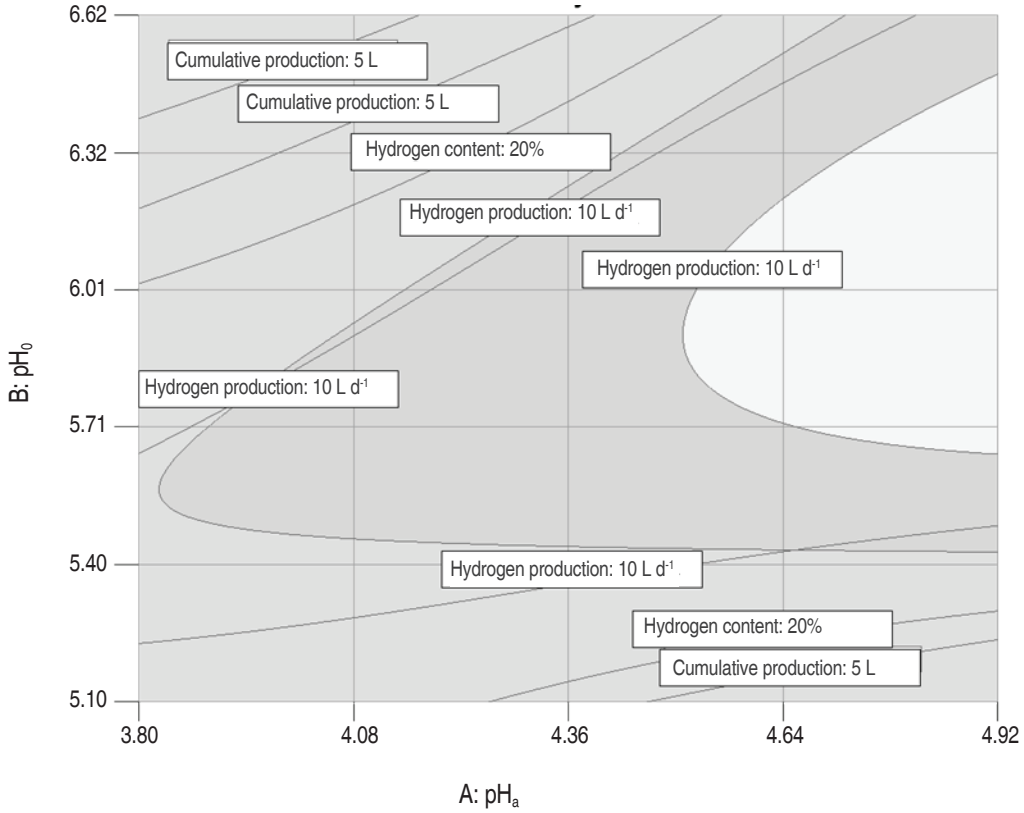

Figure 3. Superposition of the three response surfaces.

The stationary point was that in which the derivative of the model was zero. The results of the canonical analysis for the three responses show that in all cases there was a stationary point, corresponding to a saddle point. The coordinates of the stationary points and the value of the point for the hydrogen production (HP), the maximum content of hydrogen (MCH) and the cumulative production (CPH) are presented in equations 2, 3 and 4 respectively.

$$HP = (4.59; 5.8; 1.96; 37.45) = 14.49 \text{ L } H_2 \text{ d}^{-1} \quad (2)$$

$$MCH = (4.46; 5.76; 1.93; 37.97) = 43.43\% \quad (3)$$

$$CPH = (4.37; 5.78; 1.94; 37.72) = 21.38 \text{ L } H_2 \quad (4)$$

The coordinates of the stationary point for the four variables were found within the experimental region that was worked, and within the numerical values found by the numerical optimization. It was also found that the coordinates of the stationary point for the cumulative production variable (L H_2), were close to those of the points found for the maximum content of hydrogen (% H_2) and production variables (L H_2 d^{-1}), that is to say that both the numerical optimization and the graph, show a common region for the optimum.

CONCLUSIONS

It was possible to obtain hydrogen from anaerobic fermentation of organic waste without using inoculum in a batch-type bioreactor, varying the acidification time, the rate stirring, the pH of acidification, and operation pH, obtaining up to 14.4 L H_2 d^{-1}, hydrogen content up to 42.4%, and cumulative production of 20.4 L H_2. The optimization of the variables studied leads to the conclusion that for the production of hydrogen the linear variables (individual effects) have no significant influence. However, the quadratic terms for operation pH and acidification time, and the interactions between pH of acidification and operation pH, and between pH

of acidification and stirring have a statistically significant effect. The response variables were adjusted to second-order polynomial models with an R^2 between 0.83 and 0.93. In addition, it was possible to optimize the three response variables obtaining a maximum of 14.9 L H_2 d^{-1} and 49.2% of H_2 by numerical optimization, and of 22.8 L H_2 accumulated by graphical optimization.

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Evaluation of a colorant and oil extracted from avocado waste as functional components of a liquid soap formulation

Evaluación de un colorante y aceite extraídos de residuos de aguacate como componentes funcionales de una formulación de jabón líquido

doi: 10.15446/rfnam.v72n2.74573

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ABSTRACT

The present research evaluated the antioxidant, antimicrobial and *in vitro* coloring capacity of extracts with different polarity obtained from avocado seeds (*Persea americana* Mill cv. Lorena). Besides, avocado oil was extracted from the residual mesocarps of *P. americana* Mill Hass cultivar by Soxhlet methodology, and the physicochemical properties of the extracted oil, as well as its fatty acid composition, were evaluated. Both the colorant and the avocado oil were used as supplies for a liquid soap type formulation. The antioxidant activity of the colorant extracts was determined by DPPH whereby water extracts showed the highest activity among the treatments. None of the extracts showed antimicrobial activity against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The iodine value (177.52 cg I_2 g^{-1}) indicated that the avocado oil obtained has a high degree of unsaturation, and the Saponification index had a value of 190.74 mg KOH g^{-1}. The colorant extracted with NaOH (L*=0.15, a*=0.05, and b*=-0.44) from the seeds was completely stable in a liquid soap matrix at pH 6.2 during one month of storage. This analysis suggests that it has high opportunities in the soap and cosmetic industry.

Keywords:
Byproducts
Food wastes
Persea americana
Value-added product

RESUMEN

El presente trabajo evaluó la capacidad antioxidante, antimicrobiana y de colorante *in vitro* de extractos de diferente polaridad obtenidos a partir de la semilla de aguacate (*Persea americana* Mill cultivar Lorena). Además, se extrajo aceite de aguacate a partir de mesocarpios residuales de *P. americana* Mill. cultivar Hass por medio de la metodología soxhlet y se evaluó las propiedades fisicoquímicas del aceite y su composición de ácidos grasos. Tanto el colorante como el aceite de aguacate fueron usados como insumos en la formulación de un jabón líquido. La actividad antioxidante de los extractos de colorante fue analizada mediante DPPH y mostró que los extractos con agua destilada tienen la más alta actividad entre los tratamientos. No se presentó ninguna actividad antimicrobiana en los extractos evaluados ante *Staphylococcus aureus* ATCC 29213 y *Escherichia coli* ATCC 25922. El índice de yodo (177,52 cg I_2 g^{-1}) revela que el aceite obtenido tiene un alto grado de insaturaciones y el índice de saponificación fue de 190,74 mg KOH g^{-1}. El colorante extraído con NaOH (L*= 0,15 a* = 0,05 y b*= -0,44) a partir de la semilla de aguacate variedad Lorena, es completamente estable en una matriz de jabón líquido con un pH de 6,2 durante un mes de almacenamiento. Estos análisis indican que los productos obtenidos tienen altas oportunidades en las industrias de jabones y cosmética.

Palabras clave:
Subproductos
Desechos alimentarios
Persea americana
Productos de valor agregado

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The use of agro-industrial wastes has proved to be an alternative solution for obtaining bioactive compounds because most of the wastes still contains interesting compounds which can be extracted and used in different industry sectors (Palomino García *et al*., 2015). The problem of food waste is currently increasing, involving all sectors of waste management from collection to disposal (Girotto *et al*., 2015). Colombia is a large producer of avocado; it is the fifth largest avocado producer in the world after Mexico (2,029,890 t), Dominican Republic (637,690 t) and Indonesia (363,160 t) (Statista, 2017). The national production in 2017 was around 335,000 t with a planted area of 40,000 ha (Agronet, 2017).

The avocado agroindustry generates waste with great potential to be harnessed since the seeds (12-16% of the total weight of the fruit) is a source of dietary fiber, fatty acids, polyphenols, and antioxidants (Ayala-Zavala *et al*., 2011; Hiwot, 2017). On the other hand, the mesocarp or pulp has high unsaturated fatty acids content, and other valuables bioactive phytochemicals such as carotenoids, tocopherols, phytosterols, lutein, and vitamins (Dávila *et al*., 2017; López-Cobo *et al*., 2016). The previous compounds have a high biological value and can be used for new products development. According to the biorefinery concept, the processing avocado is an attractive opportunity for integrated processing of the fruit into a series of valuable products, using the waste of pulp, peel and the seed of the fruit (Dávila *et al*., 2017).

The application of a natural colorant from avocado seeds can be commercially significant; therefore, its high phenol content and other functional attributes should be explored (Dabas *et al*., 2011). Natural colorants production is an interesting alternative for the use of waste with tinting characteristics. However, the main limitation of most of these dyes for commercial applications is their chemical instability and low colorant strength. On the other side, by obtaining the avocado oil from *Persea americana* Mill. cv Hass arises as an alternative to strengthen the fruit productive chain and counteract the losses of Colombian producers due to overproduction (Serpa *et al*., 2014).

The liquid soap industry uses colorants as additives for improving its appearance (Hilgert Valderrama, 2012). The addition of fatty acids in mixes of anionic surfactants can play an important role in foam stability and ink removal from cellulose fibers (Theander and Pugh, 2003). Mixtures of synthetic surfactants, avocado, olive, mineral and castor oils have been used to improve the moisturizing properties of the liquid soaps and to prevent skin dryness (Glenn, 1996). Therefore, exploring the use of avocado waste pulps and seeds in liquid soap-like ingredients in a formulation becomes an option to generate added value to avocado agroindustry.

MATERIALS AND METHODS

Colorant extraction

Avocado seeds were harvested and classified at La Fortuna farm municipality of Mariquita, Tolima (5.255607 N, -74.998586 W). Size reduction was made with flake cuts of 3 mm and the material was dried at 55 °C during 14 h in a Memmert UF 55 oven, then they were ground and filtered with a 500-micron mesh. For colorant extraction, three solvents were used independently: distilled water, an aqueous solution of NaOH (0.5%) (Devia Pineda and Saldarriaga, 2005) and a mixture of distilled water and ethanol (1:1). A ratio of 0.05 milled seed-solvent, as well as the reflux system at a temperature of 45 °C and the extraction time of 120 min, remained constant. Extraction yields were calculated using a moisture determinant XM 60 HR.

Antioxidant activity evaluation

The antioxidant activity was measured using the DPPH method applied by Brand-Williams *et al.* (1995), which is based on the absorbance reduction of the DPPH 1, 1-diphenyl-2-picrylhydrazyl radical, measured at 515 nm.

To determine the antioxidant activity, 200 µL of each extract at different concentrations and 2800 µL of DPPH solution (0.1 mM) were mixed and taken to a dark chamber during 30 minutes at room temperature, and the absorption was measured at 518 nm in a Jenway 7305 spectrophotometer. The 2-carboxylic-6-hydroxy-2, 5, 7, 8 tetramethylchroman acid (Trolox) was used as a control. Tests were replicated three times.

The percentage of antioxidant inhibition from the extracts at different concentrations (expressed in ppm) was evaluated. The pH value of the NaOH aqueous extract

(pH 9.3), water extract (pH 5.1) and ethanol extract (pH 5.8) was neutralized to pH 7.0 The percentage of inhibition was calculated according to equation 1.

$$\%\ of\ inhibition = \frac{A - A_1}{A} x\ 100 \qquad (1)$$

A: Blank absorbance
A_1: Sample absorbance

The results were expressed as the maximum concentration of the inhibitory mean (IC_{50}), defined as the amount of antioxidant necessary to reduce the initial concentration of DPPH to 50%.

Antimicrobial activity evaluation

The antimicrobial activity was evaluated using the Kirby Bauer disk diffusion method (Hudzicki, 2009). Strains of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25992 were used for this purpose. Those strains were isolated in EMB and Baird Parker agar respectively. Three to five colonies of each microorganism were placed in a saline solution (5 ml, 0.85%) and the concentration was adjusted to the 0.5 McFarland tube (1.5×10^6 CFU mL^{-1}). Once the suspension was adjusted, it was massively seeded with a sterile brush in Mueller Hinton agar. The sterile filter paper discs (10 mm in diameter), used for the test, were previously impregnated with 0.1 mL of the extract at 5000 ppm and dried in a closed petri dish. The treatments were classified in A1 (aqueous extract; 5000 ppm and pH 5.4), A2 (NaOH aqueous extract; 5000 ppm and pH 8.2), A3 (water-ethanol extract; 5000 ppm and pH 5.5). A paper disc with chloramphenicol (10 mg mL^{-1}) was used as the positive control (A4). All experimental units were incubated at 37 °C for 24 h (Casana *et al.*, 2016). Then inhibition halos were read.

Avocado oil characterization

Matured Hass avocado was reduced in size and dried in a Memmert UF 55 plus oven at 50 °C for 12 h. Subsequently, the free fat total extraction was carried out using the Soxhlet method for 4 h with hexane (grade HPLC, Scientific). The fatty acids profile was analyzed by means of FID gas chromatography in a Thermoscientific Trace 1310 chromatographer with an Rtx-5 Restek Corporation column of 30 m long, 0.32 mm internal diameter and a film thickness of 1µm under the following conditions: injection volume 1.0 L, injector temperature 230 °C, detector temperature 250 °C, column pressure 23.04 psi, hydrogen flow in the detector 45 mL min^{-1}, air flow in the detector 450 mL min^{-1}, makeup gas flow (N_2) 45 mL min^{-1}, split flow 70.2 mL min^{-1}, split ratio 40:1.

Temperature ramp: The initial column temperature was 190 °C (during 12 min) and rose up to 220 °C with a ratio of 2.0 °C per 4.0 min. The fatty acids composition was found by comparing the peaks retention times obtained with the Fatty Acids Methyl Esters (FAMEs) standards.

Physicochemical parameters such as density was determined following the NTC 336 (ICONTEC, 2002a), Iodine index following the NTC 283 (ICONTEC, 1998a), peroxide index following the NTC 236 (ICONTEC, 1998b), refractive index following the NTC 289 (ICONTEC, 2002b), acidity index following the NTC 218 (ICONTEC, 1999), and saponification index following the NTC 335 (ICONTEC, 1998c). Tests were measured in duplicate.

Color evaluation

The extract with the highest yield was used to calculate the color parameters by using the CIELab space coordinates (L*, a*, b*) in a Konica Minolta Cr-5 colorimeter. Different concentrations of liquid dye avocado were tested (1%, 2%, and 3%) in the liquid soap matrix. The soup emulsion was stored at room temperature and exposed to light for one month. The color difference between the samples was expressed in ΔE* (Hikita *et al.*, 2001). The color variation can be estimated through the ΔE* which is determined through the L*, a*, and b* parameters (Manayay *et al.*, 2013) (Equation 2).

$$\Delta E^* = \sqrt{(L_0 - L)^2 + (a_0 - a_i)^2 + (b_0 - b_i)^2} \qquad (2)$$

ΔE*: Color variation or alteration
ΔL*: Luminosity variation between the measurements
Δa*: Variation from green to red between measurements
Δb*: Variation from blue to yellow between measurements

Liquid soap formulation, stability assessment and BOD calculation

Texapon 40 Sodium Lauryl Ether Sulphate (SLES) and distilled water were mixed and placed on a magnetic stirring plate at 200 rpm. Then sodium benzoate, cocamidopropyl betaine, glycerine, colorant, alcohol,

and salt were homogenously added in a strict sequential order. Different formulations are shown in Table 1.

Color behavior was evaluated by using the CIELab space coordinates (L*, a*, b*) in a Konica Minolta Cr-5 colorimeter (with liquid analysis accessory). The pH variation was measured with a Lovibond SD 300 potentiometer. Soap samples were stored at room temperature for one month and exposed to light.

An airtight vial was overflowed with a soap sample and incubated for 5 d to determine the biochemical oxygen demand. Dissolved oxygen was measured before and after the incubation phase and the BOD calculation results from the difference between the initial and final values of dissolved oxygen (Gender Cevallos and Arnao Ramirez, 2005). Commercial liquid soap and a sample soap (formula 4) were used to determine the BOD.

Table 1. Liquid soaps formulations.

Ingredients	Formulas' composition (%)			
	1	2	3	4
Water	54.9	53.9	52.9	52.7
Salt	4.0	4.0	4.0	4.0
Alcohol	2.0	2.0	2.0	2.0
Sodium benzoate	0.3	0.3	0.3	0.3
Texapon 40	35	35	35	35
Cocamidopropyl betaine	1.5	1.5	1.5	1.5
Glycerine	1.0	1.0	1.0	1.0
Boric acid	0.3	0.3	0.3	0.3
Avocado dye (liquid extract)	1.0	2.0	3.0	3.0
Essence	0.0	0.0	0.0	0.2
Avocado oil (waste mesocarps)	0.0	0.0	0.0	2.0

Statistical analysis

Statgraphics (Centurion version) was used to perform the analysis of variance of the color measurement, extraction performance, and antimicrobial activity. Simple linear regression was used to predict the percentage of antioxidant inhibition at different concentrations.

RESULTS AND DISCUSSION

Yields

The process of drying avocado seeds reported a yield of 27.90±0.99%. Sodium hydroxide showed the most efficient extraction with a percentage of total biomass extracted (weight/weight) of 35.72±0.43 and CIELab color coordinates of L*=0.15, a*=0.05 and b*=-0.44, followed by alcohol extraction (33.16 ±0.13 and CIELab color coordinates of L*=76.89, a*=15.49 and b*=66.74) and distilled water showed the lowest extraction yield (11.61±0.89 and CIELab color coordinates of L*=85.08, a*=5.05 and b*=50.25).

Antioxidant activity

Treatments with sodium hydroxide (T3 and T4, Table 2) showed the lowest percentage of inhibition. Samples extracted with NaOH at a concentration of 150 µg mL^{-1} and pH 9.3 showed a percentage of inhibition of 24.72%. However, at pH 7.0 the percentage of inhibition decreased to 14.03%.

Samples treated with water at pH 7.0 showed a decrease in the percentage of inhibition at all concentrations (reference value=150 µg mL^{-1}). T1 reached 51.69% (IC_{50} value=153.87 µg mL^{-1}) while T2 reached 38.96% (IC_{50} value 187.66 µg mL^{-1}). Both IC_{50} values were close to the Trolox control (84.10 µg mL^{-1}). Samples neutralized at pH 7.0 and treated with NaOH and water, showed less antioxidant activity.

Otherwise, treatments with a mixture of water and ethanol showed an increase in antioxidant activity when neutralized to pH 7.0. The ethanol extract at pH 5.8 (150 µg mL^{-1})

showed a lower percentage of inhibition (24.21%) than at pH 7 (35.01%). IC_{50} values from T5 (630.00 µg mL^{-1}) and T6 (265.67 µg mL^{-1}) were the closest to the control. Table 2 shows the results of the maximum concentration of the inhibitory mean (IC_{50}). The ANOVA (Analysis of Variance) indicates that there are statistically significant differences (P<0.05) between the treatment and the control.

Table 2. IC_{50} values of different avocado seed extracts (*Persea Americana* cv. Lorena) analyzed by the DPPH• radical scavenging method.

IC_{50} calculation of different extraction methods	µg mL^{-1}	R^2
Pattern: Trolox	84.10	98.59
T1: Water extract, Seed; pH 5.1	153.87	94.46
T2: Water extract, Seed; pH 7.0	187.66	92.39
T3: Water extract, NaOH, Seed; pH 9.3	1154.00	94.69
T4: Water extract, NaOH, Seed; pH 7.0	1284.00	95.47
T5: Water extract, Ethanol, Seed; pH 5.8	630.00	89.03
T6: Water extract, Ethanol, Seed; pH 7.0	265.67	93.78

Nagaraj *et al.* (2010) studied the antioxidant activity of methanol-water (4:1) extracts by the DPPH method and obtained a percentage of inhibition of 60.8%. In the present research, methanol was not used due to its incompatibility with the liquid soap matrix formulation; water was used instead. The aqueous extract showed 51.95% of inhibition while the Trolox control reached 80.76%. This difference is mainly due to the type of solvent used (Fu *et al.*, 2016).

Antimicrobial activity

None of the treatments showed antimicrobial activity against any of the evaluated strains; except for the chloramphenicol control. The extracts were not subjected to any type of bioactive compounds isolation or fractionation; A1 (aqueous extract; 5000 ppm and pH 5.4), A2 (NaOH aqueous extract; 5000 ppm and pH 8.2), A3 (water-ethanol extract; 5000 ppm and pH 5.5) and A4 (Chloramphenicol; 10 mg mL^{-1}). Although the use of sodium hydroxide as a solvent, showed to be the most efficient for the extraction of the dye and achieve greater dyeing power, this is not suitable for the extraction of compounds with antimicrobial activity. Therefore, in this case, the polarity of the solvent and variables such as temperature, extraction time, the nature of the matrix, the specific characteristics of the compounds and their location within the matrix must be taken into account for the optimization of the extraction of compounds with bioactive characteristics (Osorio-Tobón and Meireles, 2013).

Some studies have analyzed the antimicrobial activity from the avocado seed extracts and have defined its seeds as a good source of phytochemical components with high bioactivity (Dabas *et al.*, 2013; Nagaraj *et al.*, 2010). However, the solvent used in these studies was methanol in different conditions, and terpenoids and other bioactive compounds were fractionated.

Physicochemical characteristics of avocado oil

The drying performance of the avocado pulp was 47.41±1.22%. A paste with a rigid texture and dark green color was obtained afterward. The yield percentage of oil extraction from the dehydrated avocado pulp was 71.26±1.25%.

The iodine index is a measure of the degree of unsaturation of the fat components. There is a clear difference between the obtained index and what is established by the NTC 258 (ICONTEC, 2011). A value of 177.52 cg I_2 g^{-1} shows that the obtained oil has a high degree of unsaturation, different from the reported by Restrepo Duque *et al.* (2012) (77.85 cg I_2 g^{-1}) and Acosta Moreno (2011) (75-94 cg I_2 g^{-1}).

The peroxide index measures the fresh oil oxidation or its degree of rancidity at the time of the test (Lafont *et al.*, 2011). A value of 38.45 meq peroxide kg^{-1} reflects a high degree of rancidity. Similarly, a study that used

the same extraction method for the Hass cultivar oil reported a value of 31.66 meq peroxide kg^{-1}. However, the advanced state of ripeness and the prolonged heat treatment to which the pulp was subjected should be considered when comparing its value to olive oil with a maximum permitted limit of 20 meq peroxide kg^{-1}.

The saponification index of the avocado oil (190.74 mg KOH g^{-1}) was higher than the reported by Restrepo Duque *et al*. (2012) (175 mg KOH g^{-1}). This indicates a greater presence of low molecular weight fatty acids since the esters of this type of molecules require more KOH for saponification (Lafont *et al*., 2011). Soap and cosmetics industry demand a minimum value of 185 mg KOH g^{-1} (Lafont *et al*., 2011), this suggests that oil of this type can be used in such type of factories.

Chromatographic profile of fatty acids

The percentual sum of the oleic and linoleic fatty acids was 57.33 ± 0.33% (Table 3), a similar value was reported by Acosta Moreno (2011) (59.1%) for the Hass avocado cultivar (Acosta Moreno, 2011). This indicates that the obtained oil has a high degree of unsaturation; verified with the iodine value (177.52 cg I_2 g^{-1}). For palmitate and stearate, the sum was 24.27%. This value is higher than the reported by Acosta Moreno (2011) who obtained 16.99% for such saturated fatty acids. The difference is significant and may be due to the quality of the original raw material.

Table 3. Content of fatty acids in the avocado oil.

Fatty acid methyl esters (FAME)	Average percentage composition (%)
Laureate	0.02 ± 0.00
Myristate	0.04 ± 0.00
Palmitate	19.02 ± 0.02
Docosahexaenoic acid	0.02 ± 0.00
Linoleic	7.76 ± 0.29
Linoleic+Oleic	57.33 ± 0.32
Estearato	5.25 ± 0.04

Color and BOD assessment

The colorant obtained from the extraction with sodium hydroxide was added to the liquid soap since it showed the best performance and high colorant strength.

The color difference in all treatments can be considered small and inconspicuous since the ΔE^* values are less than 1.5 (Obón *et al*., 2009), meaning that the color given at different concentrations by the NaOH aqueous extract and avocado seed is stable. Also, the change in pH is not affected over time. According to the CIELab coordinates, the colorant has a tendency towards yellow; behavior that is linked to the concentration and reflected in the b* coordinate (where a positive number indicates yellow, and a negative number indicates blue). When the colorant concentration increased from 1% to 3%, the value of b* increased from 41.51 to 68.07. A slight tendency towards the range of red was observed in coordinate a* (where a positive number indicates red, and a negative number indicates green), this may also be closely related to the concentration since as colorant concentration increases from 1% to 3%, the a* value increases from 9.2 to 33.23. However, the L* value (where numbers between 0 - 50 indicate black or darkness and between 51 -100 indicates whiteness or clarity) decreased from 79.8 to 54.21 as the concentration increased from 1% to 3%.

In this sense, the colorant extracted with NaOH was completely stable in the liquid soap matrix (pH of 6.2) during a month of storage. The formulation containing oil at 2% also remains stable in color and pH during the same period. The ANOVA indicated that there are no statistically significant differences between the treatments for parameter ΔE^* ($P>0.05$).

The BOD of the natural colorant was 10.35 mg L^{-1}, higher than the value of the commercial soap (9.42 mg L^{-1}). This indicates that more oxygen is required to degrade the organic matter in the soap matrix by a microbial population.

Table 4. Color and pH assessment in the liquid soap for 30 days.

Liquid soap matrix treatment	Time (d)	CIELab coordinates				pH
		L*	a*	b*	ΔE*	
Soap + colorant 1%	1	79.80	9.20	41.51	0.00	6.20
	10	79.59	9.54	40.51	1.08	6.20
	20	79.14	9.37	40.08	0.65	6.21
	30	79.04	9.16	39.54	1.18	6.26
Soap + colorant 2%	1	70.23	17.95	53.92	0.00	6.20
	10	70.78	17.91	54.33	0.69	6.20
	20	70.56	17.68	53.92	0.52	6.20
	30	70.44	17.30	53.36	0.69	6.24
Soap + colorant 3%	1	54.21	33.23	68.07	0.0	6.20
	10	55.35	33.27	68.87	1.39	6.20
	20	54.80	32.67	67.78	1.36	6.20
	30	55.03	31.69	67.90	1.01	6.24
Soap + colorant 2% + avocado oil 2%	1	73.90	15.20	57.20	0.00	6.20
	10	72.83	15.20	56.47	1.30	6.20
	20	72.85	14.16	55.47	1.44	6.21
	30	73.07	13.96	54.95	0.60	6.21

CONCLUSIONS

None of the evaluated extracts from the avocado seed (Lorena cultivar) showed any antimicrobial activity against strains of *Staphylococcus aureus* ATCC 29213 or *Escherichia coli* ATCC 25922; therefore, it is necessary to evaluate the antimicrobial activity after fractionation of the avocado seed extracts and analyze the feasibility of its incorporation in liquid soap.

The dye obtained from the avocado seeds has a great potential to be used in the soap industry since it was shown to be able to confer an orange color range and is stable over time against factors such as light and pH. Additionally, this colorant presented has antioxidant characteristics.

The oil obtained from avocados that were not suitable for consumption has a significant value of unsaturated fatty acids (mainly oleic) which favors its nutraceutical and cosmetic characteristics. The avocado oil incorporated into the liquid soap presented a good behavior because parameters like the ΔE* and pH were kept constant; also no separation of the oil from the matrix was observed during the time evaluated. Therefore, this oil can have a synergistic effect on the evaluated matrix.

By using components with high biological value, it is possible to develop more environmentally friendly products that generate added value to the waste produced during the avocado processing.

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Stability of a colloidal suspension of yacon (*Smallanthus sonchifolius*) intended for spray drying

Estabilidad de una suspensión coloidal de yacón (*Smallanthus sonchifolius*) destinada al secado por aspersión

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Particle size
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ABSTRACT

Yacon (*Smallanthus sonchifolius*) is a tuber with a high content of active compounds that offer health benefits, so its productive chain seeks new alternatives to generate yacon products with added value; however, it tends to be a perishable product due to its high a_w, chemical composition, and enzymatic activity. The influence of both composition and homogenization in the colloidal stability of yacon suspensions was evaluated for later use in spray drying. Response surface methodology and a central composite design were used, considering three independent variables: homogenization time (TH) (4-6 min), xanthan gum (XG) (0.1-0.2%), and acidity (0.1-0.3%); and the following dependent variables: zeta potential (ζ), color (CIELab), viscosity (μ), stability index by spectral absorption (R), and particle size (D_{10}, D_{50}, and D_{90}). The values of the independent variables that best fit the experimental optimization criteria were: XG=0.16%, acid=0.3%, and TH=4.8 min. The values of the experimental dependent variables and the variables predicted by the quadratic model were ζ=-33.8±4.0 and -37.6 mV, μ=1143.0±93.4 and 1000 cP, R=0.45±0.1 and 0.48, D_{10}=127.8±8.2 and 138.1 μm, D_{50}=251.2±16.3 and 267.7 μm, D_{90}=424.3±28.7 μm and 463.9 μm, L*=41.7±1.4 and 41.8, a*=0.02±0.85 and 1.6, and b*=15.0±1.3 and 14.8, respectively. The colloidal suspension showed adequate physicochemical stability, favored mainly by repulsive forces, homogenization, and rheology of the continuous phase; reaching a content of total solids of 12.5%.

Palabras clave:
Tamaño de partícula
Índice de estabilidad
Viscosidad
Potencial zeta

RESUMEN

El yacón (*Smallanthus sonchifolius*) es un tubérculo con alto contenido de compuestos activos que ofrecen beneficios para la salud, por ello, su cadena productiva busca alternativas para generar productos de yacón con valor agregado; sin embargo, este tiende a ser un producto perecedero debido a su alta a_w, composición química y actividad enzimática. Se evaluó la influencia de dos factores, composición y homogenización, en la estabilidad de suspensiones coloidales a base de yacón, para un posterior uso en secado por aspersión. Se utilizó la metodología de superficie de respuesta y un diseño central compuesto, considerando tres variables independientes: tiempo de homogenización (TH) (4-6 min), goma xantana (XG) (0.1-0.2%) y acidez (0.1-0.3%). Las variables dependientes fueron: potencial zeta (ζ), color (CIELab), viscosidad (μ), índice de estabilidad por absorción espectral (R) y tamaño de partícula (D_{10}, D_{50} y D_{90}). Los valores de las variables independientes que mejor se ajustaron a los criterios de optimización experimental fueron: XG=0.16%, ácido=0.3% y TH=4.8 min. Las variables dependientes experimentales y las variables predichas por el modelo cuadrático fueron: ζ=-33.8±4.0 y -37.6 mV, μ=1143.0±93.4 y 1000cP, R=0.45±0.1 y 0.48, D_{10}=127.8±8.2 y 138.1 μm, D_{50}=251.2±16.3 y 267.7 μm, D_{90}=424.3±28.7 μm y 463.9 μm, L*=41.7±1.4 y 41.8, a*=0.02±0.85 y 1.6 y b*=15.0±1.3 y 14.8, respectivamente. La suspensión coloidal presentó una estabilidad fisicoquímica adecuada, favorecida principalmente por las fuerzas repulsivas presentes, la homogenización y la reología de la fase continua; alcanzando un contenido total de sólidos del 12.5%.

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Yacon (*Smallanthus sonchifolius*) is a tuber native from the Andes, cultivated since pre-Inca times in the inter-Andean valleys between 1500 and 2500 m.a.s.l. This tuber has multiple health benefits such as reducing of glycemic index, triglycerides and cholesterol levels, risk of colon cancer and kidney problems; help to weight control; increase the assimilation of calcium in bones; strengthening immune system response; skin rejuvenation; restoration of intestinal microflora; among others (Valentová and Ulrichová, 2003). Yacon contains between 10% and 14% of dry matter. It consists mainly of carbohydrates (70-80%), mostly fructooligosaccharides (FOS), a particular type of inulin with shorter-chain of fructans and a degree of polymerization 3–5. It also contains between 5% and 15% of sucrose; less than 5% glucose; and a significant content of antioxidants, mainly chlorogenic acid (Grancieri *et al*., 2017; Campos *et al*., 2012). Besides, other research reported between 2.4% and 4.3% of protein content and between 0.14% to 0.43% of lipids (Hermann *et al*., 1997).

The sweetening power of yacon with a minimum contribution of calories has generated great agro-industrial interest as a dietary supplement and natural sweetener (Bosscher, 2009). However, fresh roots have a short shelf-life because of their high water activity, showing values of 0.994±0.001; moisture content higher than 89.01% (Scher *et al*., 2009); type and concentration of phenolic compounds; and a high activity of polyphenol oxidase, peroxidase, and fructan 1-exohydrolase enzymes (Castillo, 2015); which all together hinder its processing and transformation.

Suspensions are thermodynamically unstable, which is directly related to different forces: van der Waals (attractive), electrostatic (repulsive), steric (absorbable macromolecules), hydration, hydrophobic, and phase separation (Piorkowski and Mcclements, 2013; Mirhosseini *et al*., 2008), being flocculation, coalescence, sedimentation, and Ostwald ripening the most common mechanisms (Larsson *et al*., 2012). In suspensions, physical-chemical instability is manifested mainly by rapid sedimentation, which can be controlled by a homogenization process that reduces particle size (Hennart *et al*., 2010) and increases the system's viscosity (Bayod *et al*., 2008). On the other hand, viscosity and total solids of the suspension affect the pumping capacity and compromises technological characteristics of the operation (Mirhosseini *et al*., 2008).

According to the above, the aim of this research was to evaluate the influence of composition and homogenization process on the stability of colloidal yacon suspensions for later use in spray drying.

MATERIALS AND METHODS

Materials

Yacon tubers harvested at commercial maturity in the Municipality of Santa Elena (Antioquia) were used. Xanthan gum (XG) was used as thickener and stabilizer. Ascorbic acid and food grade citric acid were used as regulators of the suspension acidity.

Preparation of suspensions

Batches of 3 kg of yacon suspensions (YS) were prepared. Tubers stored at 25 °C were disinfected in 50 ppm NaClO solution, then they were peeled, chopped and immediately passed through an FPSTJE317 Oster® Juice extractor, where insoluble partially disintegrated material was separated from its extract. Subsequently, both were poured into a container and the first homogenization was done using an L5M-A Silverson vertical homogenizer at maximum speed (10200 rpm) for 10 min, keeping the container in a water bath at approximately 25 °C and adding the mixture of ascorbic and food grade citric acid (ratio 1:1); afterward, XG was slowly added. The resulting suspension, in this preliminary phase, was submitted to a second homogenization using an ST REGIS 3DD13-2941 Chicago USA high-pressure piston homogenizer, at 1200 psi during the time set in the experimental design to reduce particle size and improve YS stability.

Determination of the dependent variables

The zeta potential (ζ) was determined in the yacon suspension diluted in deionized water (1:100), using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., Worcester, UK) at 25 °C (Rezvani *et al*., 2012). The spectral absorption stability index (R) was determined in a UV-Visible spectrophotometer (Thermo Scientific Evolution 60), from absorbance ratio of 800 and 400 nm ($R=A_{800}/A_{400}$) (Song *et al*., 2002) applied to diluted YS (1:100). Viscosity (μ) was determined

on a rheometer (Brookfield DV-III Ultra, Brookfield Engineering Laboratories, Inc., USA) coupled with a Brookfield thermostatted bath model TC-502, RV5 spindle, speed from 0.01 to 100 rpm and reported at 100 rpm (Mirhosseini *et al*., 2008). Particle size was reported as percentiles D_{10}, D_{50}, and D_{90}; using a Mastersizer 3000 (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom) (Millqvist-Fureby and Smith, 2007). The color was determined with the CIELab coordinates, where L* measures luminosity, a* measures green (-) to red (+) chromaticity, and b* measures blue (-) to yellow (+) chromaticity; using an SP64 - X-Rite spectrophotometer, illuminant D65, and observer of 10°.

Statistical design

Response surface methodology with a central composite design (α=1) was used, considering as independent variables: homogenization time (TH) (4-6 min), XG added (0.1-0.2%) and acid (0.1-0.3%). The dependent variables were ζ, R, μ, color (coordinates CIELab), and particle size (D_{10}, D_{50}, and D_{90}). All the variables were measured by triplicate. Results were analyzed through an Analysis of Variance with a confidence level of 95%, using Sofware Design Expert 8.0 (Stat-Ease, Inc USA). Significant differences and correlations were made by least significant difference (LSD) (level of significance $\leq$5%) and Pearson correlation, respectively. Experimental optimization was performed considering the statistical results obtained, and according to criteria that favored suspension stability: maximize (L*, a*, b*), minimize (ζ, D_{10}, D_{50}, and D_{90}), and μ set at 1000 cP.

Mathematical modeling

A mathematical model was made using a polynomial model of order 2 (equation 1), where Y is the dependent variable, β_0 is the constant, β_A, β_B, and β_C are linear coefficients; β_A^2, β_B^2, and β_C^2 are quadratic coefficients; β_{AB}, β_{AC}, and β_{BC} are factors interaction coefficients. The adequacy of models was carried out using the lack of fit test and regression coefficient (R^2).

$$Y = \beta_0 + \beta_A\beta_B B + \beta_C C + \beta_A^2 A^2 + \beta_B^2 B^2 + \beta_C^2 C^2 + \beta_{AB} AB + \beta_{AC} AC + \beta_{BC} BC \quad (1)$$

Experimental values obtained from three additional experiments were compared with optimal conditions of the process in order to verify the accuracy of dependent variables obtained by the mathematical models.

RESULTS AND DISCUSSION

Table 1 shows mean values and standard deviation of YS dependent variables according to experimental

Table 1. Results of central composite design for YS development.

Run	XG (%)	Acid (%)	TH (min)	STS (%)	ζ (mV)	μ (cP)	R
1	0.15	0.3	5	14.5	-37.5±2.7	898±13	0.470±0.038
2	0.10	0.3	6	14.5	-32.6±5.0	1160±18	0.516±0.018
3	0.15	0.2	5	14.4	-30.3±1.2	1276±12	0.544±0.029
4	0.15	0.2	5	14.4	-35.7±2.0	1217±15	0.544±0.018
5	0.20	0.3	4	14.6	-38.0±1.4	1455±10	0.446±0.025
6	0.15	0.2	6	14.4	-30.5±2.3	1380±15	0.506±0.076
7	0.15	0.2	5	14.4	-31.6±1.2	1370±13	0.512±0.085
8	0.15	0.1	5	14.3	-41.3±0.6	1385±15	0.518±0.015
9	0.10	0.1	4	14.2	-32.6±1.4	1149±13	0.552±0.012
10	0.10	0.2	5	14.3	-25.6±0.7	912±10.6	0.480±0.019
11	0.20	0.1	6	14.3	-38.4±1.1	1652±15	0.607±0.014
12	0.20	0.2	5	14.4	-36.1±1.0	1581±13	0.526±0.037
13	0.15	0.2	5	14.4	-31.8±2.3	1116±16	0.551±0.046
14	0.15	0.2	5	14.4	-34.0±1.2	1195±17	0.537±0.013
15	0.15	0.2	4	14.4	-35.0±1.1	931±12.0	0.498±0.048

STS: Suspension Total Solids

Table 1. Continuation

Run	D_{10}	D_{50}	D_{50}	L*	a*	b*
		(µm)				
1	129.3±2.5	266.3±0.6	472.6±3.2	41.9±1.3	1.9±0.1	14.9±0.7
2	137.0±2.0	227.0±0.8	388.3±4.5	46.1±0.4	-0.2±0.0	8.4±0.3
3	142.0±2.6	261.0±2.3	441.0±3.6	41.7±0.6	2.3±0.2	12.9±0.5
4	145.0±0.6	263.7±1.8	453.6±5.8	40.5±0.8	2.4±0.3	12.7±0.2
5	164.0±1.0	278.7±0.4	447.7±5.5	44.3±2.2	-0.4±0.3	10.3±1.0
6	139.3±6.0	262.3±4.5	444.3±3.1	40.4±0.9	2.8±0.0	11.7±0.5
7	139.5±0.0	262.5±2.6	545.0±4.6	39.9±0.4	2.3±0.2	11.8±0.1
8	131.0±1.0	264.0±2.0	463.3±5.5	41.6±0.8	0.3±0.0	13.5±0.5
9	145.6±2.3	290.6±1.5	516.6±2.5	40.8±1.1	-0.01±0.1	13.2±0.4
10	115.3±0.6	237.6±1.2	429.3±3.2	41.4±0.2	2.3±0.1	10.9±0.1
11	157.0±0.3	236.7±0.5	332.0±1.7	39.6±0.1	-0.1±0.1	9.8±0.4
12	131.0±1.0	273.0±3.6	521.6±5.5	43.6±1.0	0.9±0.1	12.8±0.8
13	125.0±1.0	253.3±1.5	463.0±3.4	40.6±0.3	2.0±0.0	16.2±0.6
14	126.3±1.2	253.6±1.2	428.0±3.6	39.7±0.2	1.9±0.0	16.5±0.4
15	145.6±1.5	288.3±2.9	511.6±5.1	38.8±0.3	2.2±0.2	14.2±0.4

design. The ζ of YS varied between -25.1 and -45.4 mV, identifying a predominance of negative charges outside the layer of coions, and a high density of positive charges in the double electric layer. Absolute values above 25 mV indicated that the particles were highly charged, which favors electrostatic repulsion forces and therefore, better stability of YS (Dickinson, 2009; Estevinho *et al*., 2014). Statistically significant (P<0.05) response surface graphs of dependent variables are shown in Figure 1. On the other hand, Table 2 shows significant effects (P<0.05) of each dependent variable concerning independent variables and their interactions.

Table 2. Main effects of factors and interactions on YS dependent variables.

Variable	Quadratic model (P<0.05)	Lack of adjustment (P>0.05)	Intercept	Main effects			Interaction			Quadratic effects		
				A	B	C	AB	AC	BC	AA	BB	CC
ζ	0.038	0.546	-33	-5.3*	1.9	2.2	2.4	1.8	-2.5	2.6	-5.96*	0.7
µ	0.036	0.138	1190.9	334.7*	-240.7*	227.2*	175.2	-194.1	135.2	110.6	3.3	21.8
D_{10}	0.352	0.151	131.4	7.8	-0.8	-3.2	0.7	-0.4	-1.7	-3.1	3.9	16.2
D_{50}	0.013	0.067	261.9	17.7*	1.2	-13.0*	13.3*	6.5	18.3*	-10.5	-0.6	9.5
D_{90}	0.297	0.407	476.8	46.2	4.7	-33.7	27.3	7.8	77.5	-14.7	-22.2	-12.2
R	0.286	0.08	0.5	0.025	-0.025	0.005	-0.028	0.023	0.028	0.006	-0.004	0
L*	0.009	0.827	40.5	1.1	0.1	0.8	0.7	-2.4*	1.8*	2.0*	1.2*	-0.9
a*	0.004	0.054	2.3	-0.7*	0.8*	0.3	0.3	0.9*	-0.6	-0.9*	-1.4*	-0.1
b*	0.327	0.859	14.1	0.9	0.7	-1.2	0.1	1.8	1.3	-2.4	-0.005	-1.2

A: XG; B: Acid; C: TH; *: Significant effect (P<0.05).

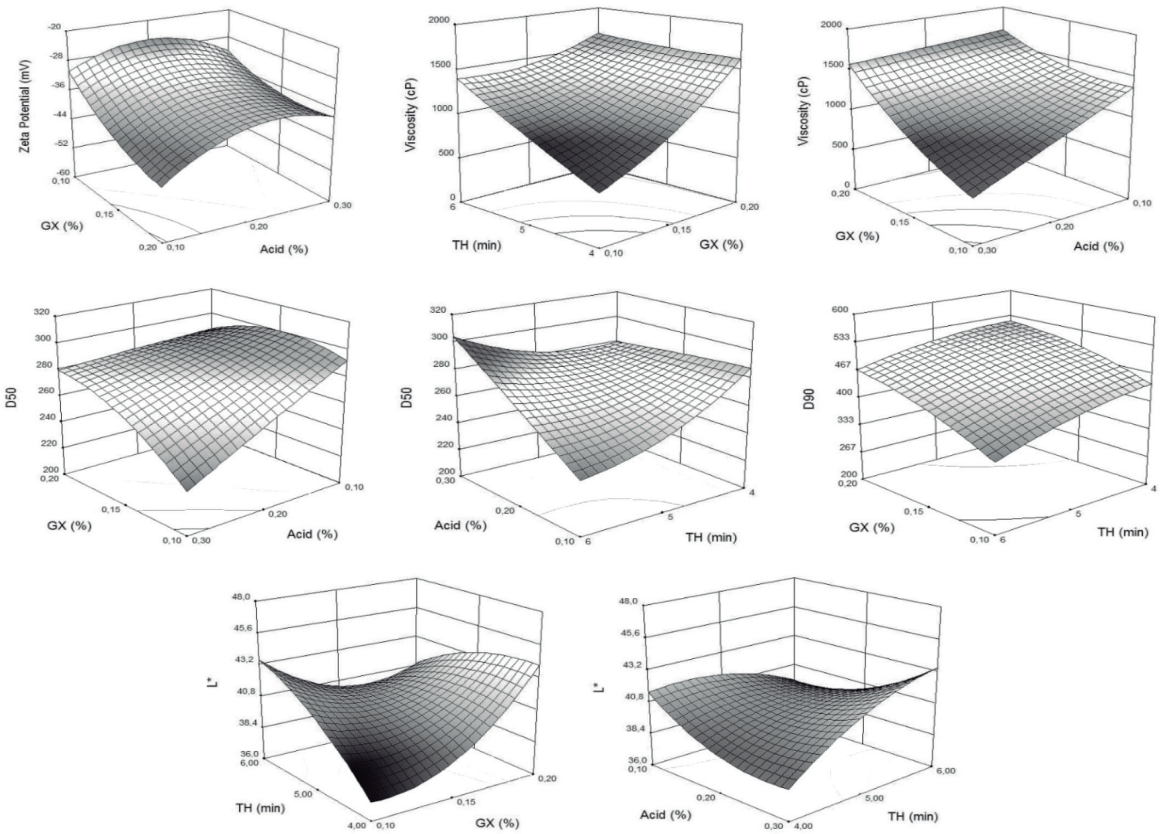

Figure 1. Response surface graphics of dependent variables as a function of independent variables.

As can be observed in Figure 1, the increase of XG confers thickening and stability to YS. It makes ζ more negative and influences net charge of YS particles, favoring repulsive forces between particles (Figueroa *et al.,* 2016). Besides, because of the hydrophilic nature of XG, it confers steric stability (Flatt and Bowen, 2003). Similar cases have been reported in tamarillo drinks (Figueroa *et al*., 2016), where the inclusion of XG (0.025%) and carboxymethylcellulose, (0.050%) modified ζ to -17.9±1.1 and -30.5±1.8 mV, respectively. The same situation has been reported in orange oil emulsions adding thickeners such as XG, gum Arabic, and gelatin with positive effects on the system colloidal stability (Mirhosseini *et al*., 2008).

Ascorbic and citric acid showed a quadratic effect on ζ (curvature behavior), being less negative at 0.2% and more negative at 0.1%. These changes modify double layer repulsion forces and electrostatic interactions (ion-particle interactions); as well as ion-ion interaction and YS rheology (Flatt and Bowen, 2003). The pH of the YS varied between 3.8 and 4.9; whereas, the acidity varied between 0.1 and 0.3%. These dependent variables affect ζ because they influence the action of the XG and ionic strength of YS. In orange oil emulsions, it has been reported that the decrease in pH was correlated with the increase in emulsion stability and ζ magnitude (Mirhosseini *et al*., 2008).

μ showed a tendency to increase when XG addition increases, as well as with TH. Probably because when XG is solubilized undergoes a conformational transition from a double helix to a complex aggregates by hydrogen bonds and polymer intricacy (Jayme *et al*., 1999, Niu *et al*., 2017) forming a more complex network of polymers that includes FOS, which translates into an increase in the apparent viscosity (μ_a) and pseudoplastic behavior of the colloidal system.

This behavior has been observed in coconut-based emulsions (Lucas *et al*., 2018) and orange beverage emulsions (Mirhosseini *et al*., 2008). According to Stoke's law, sedimentation or cremation rate of colloidal systems is inversely proportional to the viscosity of the aqueous phase; therefore, XG contributes to improving YS thermodynamic stability (Mirhosseini *et al*., 2008). The effect of TH on the μ of YS is marked mainly when the colloidal system contains low XG levels, which could be due to the formation of aggregates or flocs that confer an "elastic" behavior that is typical of suspensions known as physical gels (Castro *et al*., 2013).

The effect of TH when the concentration of XG was the maximum (0.2%) was less significant in the change of μ, which is corresponding to the reported by some authors, who rather attribute that change to the contribution of insoluble solids of yacon than to homogenization degree. Values of the elastic modulus (G') between 100-150 Pa, 200-370 Pa, and 550-750 Pa have been reported for concentrations of 70, 80, and 100% of yacon pulp, respectively (Castro *et al*., 2013). μ presented enough coherence concerning the acid added to low XG levels, decreasing with the increase of acid in YS. H^+ ions interaction decreases the ionic strength of YS and the electrostatic repulsive forces between –COO– groups located in the side chain of XG, which leads to the chains' expansion and a decrease of the μ (Brunchi *et al*., 2016).

Particle size in colloidal systems is an important parameter associated with stability by phase separation phenomena (Larsson *et al*., 2012). Generally, particle sizes in YS varied among D_{10} (125-164μm), D_{50} (227-292μm), and D_{90} (332-550μm), which denotes high particle sizes and high polydispersity. The high content of insoluble fibers, which would be interacting with soluble components (inulin-type fructans) and other biopolymers, modify YS rheological properties (Bayod *et al*., 2008; Sharma *et al*., 1996). This situation is consistent with the statistical results obtained in D_{10} and D_{90} percentiles which did not show significant effects (P>0.05), nor to the interactions of independent variables considered; although there is a tendency to favor smaller sizes with higher TH.

It is observed that an increase of XG has a marked effect on the increase of D_{50}, which is directly associated with higher μ of YS, causing an increase in resistance to homogenization. The lack of homogenization does not favor the reduction in the size of insoluble fibers (Raghavendra *et al*., 2006). Additionally, the effect of a higher TH is reflected in lowering D_{50}, mainly when less acid is added (0.1%) to YS. A similar behavior (<D_{50}) is experienced by XG-Acid interaction at lower levels of XG and higher acid addition (0.3%). The presence of H^+ ions has been commented previously and also its effect on XG action; additionally, these results suggest there is an interaction between acid level and TH that could confer an increase in D_{50} because of YS elastic behavior, promoting the formation of small particles and aggregates (Castro *et al*., 2013). In the same way, other authors have reported that homogenization (speed and TH) reduces polydispersity of particles and thereby increases elastic properties (Luckham and Ukeje, 1999).

R is based on the dispersion properties of light, which is related to the average particle size and wavelength (A_{800}/A_{400}), low values denote better stability of the colloidal system (Cortés, 2004). Average values of R varied between 0.446 and 0.607, without reaching significant differences (P>0.05) regarding the evaluated factors, for the same polydispersibility mentioned before. In general, it is highlighted that YS was stable and did not show phase separation after 24 h, which is favored by continuous phase with high μ and the effect of high ζ (Dłużewska *et al*., 2006). On the other hand, some authors have reported on emulsified drinks with orange oil (Mirhosseini *et al*., 2008), the interaction of XG and gum arabic had no greater effect on R.

Color parameters of fresh yacon were L*=41.2±1.2, a*=3.1±0.4, and b*=10.5±0.6. In YS the dominant color was between cream and orange, varying their average values of L*, a*, and b* between (38.8 and 43.6), (-0.4 and 2.8), and (8.4 and 16.5), respectively. Yellowish and reddish tones in products are attributable to the content of carotenoids present in yacon roots *(*Franco *et al*., 2016); although b* chromaticity did not show significant differences (P>0.05) because of the effects of the evaluated factors, nor for their linear or quadratic interactions. Color parameters L* and a* of YS were the most affected statistically, varying between (38.8 and 46.1) and (-0.4 and 2.4); however, when changes in a* chromaticity are so low, they are not perceptible to the human eye. On the other hand, b* chromaticity was not significant

concerning the factors studied; it could be due to some variations in yacon roots' color because of its composition, ripeness, among others. L* represents the parameter to be controlled in YS, in which an effect of the XG-TH interaction was observed; the greatest clarity (>L*) was reached in combinations of 0.2% - 4 min and 0.1% - 6 min. This result implies an encapsulating action of the biopolymer, protecting bioactive compounds responsible for the yacon's color; such as betalains, polyphenols, carotenoids, and antoxanthines; that works against oxidative processes and factors of the surrounding environment such as humidity, pH, light, and heat. A similar situation occurs with the TH-Acid interaction, favoring L* in combinations of 0.3% - 4 min and 0.1% - 6 min.

The fit of response surface models and statistical significance of mathematical models was evaluated through an ANOVA, lack of fit test (LOF), and regression coefficients (R^2) which are presented in Tables 2 and 3.

Quadratic regression models with one (P<0.05) and those with the best regression fit (R^2>0.9) were ζ, μ, D_{50}, L * and a*, showing good agreement between results predicted by the model and those observed within the experimentation range. R, D_{10}, D_{90}, and b* variables presented values of R^2<0.9, indicating that models were not sufficiently predictive, maybe due to several factors not controlled in the research: variation in color of yacon roots, composition, ripeness, and aggregation of particles during YS repose state after homogenization, among others.

Table 3. Regression coefficients, R^2 and probability values of lack of fit of the models for dependent variables.

Regression coefficients	ζ (mV)	μ (cp)	R	D_{10}	D_{50}	D_{90}	L*	a*	b*
					(μm)				
β_0: constant	1.73	579.18	1.1	498.36	773.8	1029.44	19.26	-4.66	4.44
β_A	-693.73	5815.49	-1.36	545	425.98	805.16	-4.63	-7.02	118.21
β_B	310.24	-14558.64	-0.64	-97.92	-1279.4	-3762.1	-156.58	87.49	-60.21
β_C	-5.35	320.87	-0.18	-160.4	-164.44	-90.62	13.4	-0.72	3.12
β_{AB}	471.5	35049.5	-5.5	149.5	2667	5466.5	131	57	18.5
β_{AC}	35.55	-3881.65	0.45	-8.35	130	156.75	-47.2	18.6	36.25
β_{BC}	-24.73	1352.48	0.28	-17.48	183.3	775.03	18.25	-6.15	12.98
β_A^2	1054.59	44253.9	2.35	-1254.8	-4186.9	-5862.7	785.76	-370.94	-947.88
β_B^2	-595.85	330.47	-0.41	386.29	-63.74	-2215.7	117.94	-143.24	-0.47
β_C^2	0.72	21.8	0.006	16.2	9.53	-12.16	-0.92	-0.05	-1.24
R^2	90.77	91.02	75.57	72.49	94.28	75.07	95.08	96.46	73.65

The criteria for experimental optimization of YS were set considering minimizing ζ, higher density of negative charge to favor repulsive forces; minimize R and percentiles D_{10}, D_{50}, and D_{90}, characteristic of suspensions with better stability; set μ of suspension in 1000 cP, design criterion of the pilot spray dryer Vibrasec PASLAB 1.5; maximize the color parameters L*, less browning; a* and b*, greater red and yellow pigmentation respectively. These criteria allowed selecting optimal independent variables: XG=0.16% w/w, Acid=0.3% and TH=4.8 min.

Table 4 shows de comparison of experimental values of YS-dependent variables, obtained from three replicates to optimal conditions, with the theoretical values obtained from the mathematical models observing a good approximation between them with a relative mean error values (RME) lower than 10%.

Table 4: Experimental and theoretical values of YS dependent variables.

Variable	Experimental value	Theoretical value	RME
ζ (mV)	-33.83±4.02	-37.6	0.88
R	0.45±0.06	0.48	0.8
μ (cp)	1143±93.41	1000	0.76
L*	41.74±1.38	41.82	0.13
a*	0.02±0.85	1.58	8.41
b*	14.97±1.35	14.8	0.39
D_{10} (μm)	127.83±8.23	138.15	0.38
D_{50} (μm)	251.17±16.34	267.68	0.42
D_{90} (μm)	424.33±28.74	463.88	0.58

CONCLUSIONS

Yacon is a tuber with health benefits; therefore, due to its industrial potential, it is necessary to generate value for its agricultural chain. The use of statistical tools from an experimental optimization allowed the development of a colloidal suspension based on yacon, stable and adequate from the technical point of view, intended for spray drying processes, which represents an effective alternative of agro-industrialization. Electrostatic forces of the colloidal system favor the stability of YS; XG increases the viscosity of the system, forming a more complex network of polymers that includes soluble and insoluble solids of yacon. Moreover, there are XG-Acid interaction that affects the electrostatic forces of the colloidal system by the H^+ ions action that decreases the ionic strength of YS. In addition, the homogenization process affects the rheology of the system, being an important factor in phase separation phenomena. Mathematical modeling of dependent variables presented a good fit with relative mean error values <10%, which guarantees the right prediction of dependent variables.

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Evaluation and modeling of the properties and antioxidant characteristics of a new potato variety (Primavera) during storage at 4 °C

Evaluación y modelado de las propiedades y características antioxidantes de una nueva variedad de papa (Primavera) durante su almacenamiento a 4 °C

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ABSTRACT

Potatoes are one of the crops with the greatest influence worldwide, and Colombia is the most important exporter of "Criolla" or diploid potato. Universidad Nacional de Colombia has developed varieties of new diploid potatoes with high antioxidant properties and colored flesh: Primavera, Paola, Violeta, Milagros, and Paysandú. The aim of this research was to characterize and evaluate the stability of physicochemical properties of the raw potato cv Primavera during storage at 4 °C. It was used the potato variety Primavera grown in Santa Elena, Antioquia, during season mayo-august 2016. The evaluated properties were the antioxidant capacity (DPPH and ABTS), phenolic compounds (Folin-Ciocalteu method), moisture, texture in whole tuber and slices, and color in the pulp (CIELab). Samples were stored in bags at a constant temperature of 4 °C and were evaluated for 0, 7, 14, 21, and 30 d. Polynomial regression was performed for each variable vs time. In general, properties for potato variety Primavera did not show a defined trend; otherwise, they were fluctuating; this may be associated with various factors such as primary production and the interaction of physic-chemical phenomena of the matter with its environment. Potato presented an important content of antioxidant compounds compared with other varieties (ABTS: 2.89→2.94 mg Trolox g^{-1}; DPPH: 2.33→1.48 mg Trolox g^{-1}; phenolic compounds: 6.09→6.27 mg gallic acid equivalent g^{-1}). The "criolla" potato cv Primavera has a lot of important antioxidant properties which could confer it an agro-industrial potential in a short and medium term.

Keywords:
Antioxidants
New potato variety
Phenolic compounds
Storage tubers
Shelf-life

RESUMEN

La papa es uno de los cultivos con mayor influencia a nivel mundial y Colombia se destaca por ser el primer exportador de papa criolla o diploide. Nuevas variedades de papa diploide con propiedades antioxidantes y coloración en su pulpa han sido desarrolladas por la Universidad Nacional de Colombia: Primavera, Paola, Violeta, Milagros y Paysandú. El objetivo del presente estudio fue caracterizar y evaluar la estabilidad de las propiedades fisicoquímicas de la papa variedad Primavera, en estado fresco, durante su almacenamiento a 4 °C. Se utilizó papa variedad Primavera, cultivada en Santa Elena, Antioquia, durante los meses de mayo-agosto de 2016. Las propiedades fueron evaluadas mediante la capacidad antioxidante (DPPH y ABTS), fenoles totales (método de Folin-Ciocalteu), humedad, textura en la papa entera y en rodajas, y el color en la pulpa (CIELab). Las muestras fueron almacenadas en bolsas a una temperatura constante de 4 °C y fueron analizadas en los tiempos de control 0, 7, 14, 21 y 30 d. Se realizó una regresión polinomial para cada variable en función del tiempo. En general, las propiedades evaluadas de la papa variedad Primavera no presentaron una tendencia definida, por el contrario, fueron fluctuantes, lo cual podría estar asociado a diversos factores desde la producción primaria; así como a la interacción de fenómenos químicos y físicos de la materia con su entorno. La papa presentó un contenido importante de compuestos antioxidantes comparada con otras variedades (ABTS: 2,89→2,94 mg Trolox g^{-1}; DPPH: 2,33→1,48 mg Trolox g^{-1}; fenoles totales: 6,09→6,27 mg ácido gálico equivalente g^{-1}). La papa criolla variedad Primavera es un alimento que posee importantes propiedades antioxidantes, lo cual le podría conferir un potencial de agro-industrialización a corto y mediano plazo.

Palabras clave:
Antioxidantes
Nueva variedad de papa
Compuestos fenólicos
Almacenamiento tubérculos
Vida útil

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Potato is one of the crops with the highest production worldwide. China is one of the biggest producer, with 95 million tons produced annually. In Colombia, 3 million tons are produced annually (FAOSTAT, 2016). Potatoes are a desired and targeted product because of its high nutritional value, presenting high content of starch and micronutrients. Besides, it is versatile during steaming, allowing to prepare different recipes with it.

An advantage of *Solanum phureja* Juz. et Buk (Andean ancient species) is that have a better capacity to be pollinated with other potato species, resulting in a wide range of wild-type species from the Andes. This tuber has been used widely in programs of crop genetic improvement (Juyó *et al*., 2011).

With this selection process, diploid potatoes with antioxidant properties and resistant to diseases, such as powdery scab and late blight, have been obtained (Singh and Kaur, 2016). The potato variety Primavera is part of these new varieties; it was developed at Universidad Nacional de Colombia, and its main characteristics is a colored peel and pulp with red and purple colors (Figure 1) (ICA, 2016).

Figure 1. Potato (*S. phureja* cv Primavera).

One of the most important attributes in colored potatoes is the high antioxidant content including phenolic compounds like anthocyanins (Kita *et al*., 2015). Although this kind of potato is a versatile gastronomic alternative, because of its color and bioactive compounds, this product has a short lifespan after being harvested (Molina *et al*., 2015). This short lifespan limits its storage for long periods; therefore, fast processing is required, representing a problem for industries that likely discard them because it does not meet the current quality standards in parameters like texture, dry matter and color (van Dijk *et al*., 2002a).

Considering the lifespan characteristics of potato variety Primavera, the aim of this research was to characterize a new potato variety (Primavera), in a fresh state, in terms of moisture, phenolic compounds, color, texture, antioxidant activity, and evaluate its stability during storage at 4 °C.

MATERIALS AND METHODS

Raw material

Potato (*S. phureja* cv Primavera), which is a diploid potato, was collected from an in-field and non-technified crop in Santa Elena, Antioquia. The place is located at an altitude of 2300 m.a.s.l., and presents an average temperature of 14.5 °C and average relative humidity of 89% during may-august, 2016. The potatoes were processed the fifth day after harvest, previously cleaned and disinfected with water and organic acids Citrosan® to eliminate pathogen microorganism (1 mL L^{-1}); this day was considered as the storage day 0.

A completely randomized experimental design was used; where samples were evaluated for 0, 7, 14, 21, and 30 d. This elapse let evaluate the stability of the whole potato in real time. The potatoes were stored in low-density polyethylene bags with holes to enable air exchange in a refrigerator at 4 °C and local atmospheric pressure (640 mm Hg). The amount of potatoes stored were 1 kg each lot.

To determinate the antioxidant activity, moisture, and total phenolic compounds the potatoes were chopped and grind in order to not have differences between the measurements.

Methods

Moisture content. 2 g of macerated potato were weighed and taken to a forced convection oven at 105 °C for 5 h; the moisture content was reported as the loss of weight in moisture base (AOAC, 2005).

Antioxidant activity. The measurement of antioxidant activity was done with indirect methods of DPPH (α, α-diphenyl-β-picrylhydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid). The antioxidants extraction was done by mixing 3.5 mL of Methanol reactive grade with 3 g of potato variety Primavera previously macerated with peel; then the sample was sonicated for 20 min and centrifuged for 20 min at 9000 rpm (Repo de Carrasco and Encina Zelada,

2008). The extracts were covered from light and stored at 2 °C until used.

The ABTS method was reported by Re *et al.* (1999). For it, 20 µL of methanolic extract was taken and mixed with 2000 µL of ABTS radical. After 7 min in darkness, the data of absorbance at 734 nm was registered, and the concentration was reported in mg Trolox g^{-1} dry basis. A calibration curve was previously made to determine the percentage of inhibition. It was made from 9 points of concentration of trolox in the cell between 0 and 16 µM Trolox, and this yielded a zero-order equation (Y=4.2163X+1.2276 with R^2=0.9956)

The DPPH method was done according to the methods reported by Brand-Williams *et al.* (1995). 20 µL of methanolic extract was mixed with 1980 µL DPPH radical and were registered at an absorbance of 517 nm after 30 min in darkness, the results were reported in mg Trolox g^{-1} dry basis. A calibration curve was previously made to determine the percentage of inhibition. It was made from five points of concentration of trolox in the cell between 0.0016 and 0.0046 mg Trolox mL^{-1}, and this yielded a zero-order equation (Y=2398X-23.654 with R^2=0.9865)

Total phenolic compound. The measurement was done according to the methods reported by Wang *et al.* (2016); the extraction of fresh potato phenols was performed by mixing 3 g of potato with 4 mL of Methanol: Water (60:40) solution. The mix was sonicated for 20 min and centrifuged for 30 min at 9000 rpm; 20 µL of the extract was taken and mixed with 480 µL of distilled water, 1250 µL of calcium carbonate 20% and 250 µL of diluted Folin-Ciocalteau reagent (1:1). The absorbance was read at 760 nm, after 2 h of reaction in darkness. A calibration curve was previously made from 13 points of concentration of GAE in the cell between 0 and 24 mg GAE L^{-1}, and this yielded a zero-order equation (Y=0.0186X+0.00032 with R^2=0.9939). The results were reported as mg GAE g^{-1} dry basis.

Texture. Fracture and resistance assays were performed using a texture analyzer TA-XT2i Stable Micro Systems (SMS) and the Software Texture Exceed, version 2.64. During measurements, whole and longitudinal sliced potatoes of 2 mm thickness were placed on a reference surface SMSP/35, and an awl with a spherical terminal (SMS P/0.25s) was used to measure fracturability of the chopped potatoes; an awl SMS P/2 was used to measure the whole potato.

Color. The color measurements were determined in the center of pulp (reddish zone) with a spectrophotometer X-Rite (SP64 model), which works with CIELab coordinates, illuminate D65, 10º observer, specular included (SPIN). The ΔE quantity was computing as follow:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

Where L_0^*, a_0^*, and b_0^* are the parameters of color in CIELab coordinates at time 0.

Statistical analysis. The measure for each quality attribute of potato during each time of control was performed as follow: moisture, a_w, antioxidant capacity, total phenolic compounds (three samples, one measure/sample); color and texture (six samples, one measure/sample). The polynomial regression was used to estimate the performance of each quality attribute versus the time. The experimental error was partitioned in lack of fit and pure error (Walpole *et al.*, 2012). For the parameter of color (L*, a*, b*) a multi-response model with the correlated error was used, and a secondary parameter ΔE based on predicted values was calculated (Hadfield, 2010). All statistical analyses were performed in the R environment (R Core Team, 2016) and the packages MCMCglm (Hadfield, 2010), and coda (Plummer *et al.*, 2006) were used.

RESULTS AND DISCUSSION

The moisture values for the potato variety Primavera during storage were adjusted to a cubic model (Table 1). As can be seen in Figure 2A, a general water loss occurred in the tuber throughout storage, a change of direction in the slope indicated a gain of moisture of the product at 14 d. Moisture was fitted to a cubic model. For both, the whole potato and the slices, the model that best fit was the quadratic one. Similar results were reported by Rivero *et al.* (2003) for potatoes from Tenerife, which lost water after 20 weeks of storage at 12 °C. This process of water loss happens through the periderm layer because of its high permeability (Singh and Kaur, 2009). Mass transfer occurs because of the difference in water chemical potential that exists between the potato and the environment and for breathing processes and transpiration of the tuber

(Kaya *et al*., 2016). Near 14 d, a change of direction in the slope indicated a gain of moisture of the product, this can be due to changes in environmental conditions, as relative moisture, which was not controlled during the experiment.

The initial values of moisture of fresh potato variety Primavera were similar to those reported by Bártová *et al.* (2015), (81%) in fresh *S. phureja* diploid potatoes, and by Cerón-Lasso *et al.* (2018), who reported moisture between 72.06% and 77.24% for genotypes G2589, G2585, G1997, G0204, G2599, and G1781 of native diploid potatoes (*Solanum phureja*).

In the case of the whole potato, a slight increase in the slope F/D is observed until 7 d. Figure 2 shows an increase in the slope F/D until 14 d, which is followed by a subsequent decrease in the values for the sliced potato.

Table 1. Coefficients of the polynomial that models each of the quality attributes of the potato variety Primavera and its respective adjustment coefficient (R^2).

Property	Coefficients				R^2
	β_0	β_1	β_2	β_3	
L	37.46**	0.410	-0.070	0.0020	0.99
a*	18.52**	1.610*	-0.180**	0.0040**	0.99
b*	5.24+	-0.230	0.005	0.0001	0.73
ΔE	----	----	----	----	0.99
ABTS	2.70*	-0.298	0.032+	-0.0007+	0.92
DPPH	2.30***	-0.249***	0.015***	-0.0003***	0.96
Phenolic compounds	6.43***	-0.613+	0.050+	-0.0010+	0.39
Moisture	80.79***	1.100**	0.064**	-0.0010*	0.99
Texture (Whole tuber)	4.42***	0.072+	-0.005**	----	0.97
Texture Slices	0.12	0.085*	-0.003*	----	0.85

+, *, **, *** represent a significance level of 0.10, 0.05, 0.01, and 0.001, respectively.

The increase in the slope F/D for the whole potato indicates a less turgor of the tissue. This behavior would be due to the degradation of pectins and changes in monovalent and divalent ions in the cell wall of the tuber, which causes a decrease in the cell adhesion and therefore a lower resistance to breaking by puncture (van Dijk *et al*., 2002b). This result was similar to the one reported by Solomon and Jindal (2005) for crossed potatoes stored at 24 °C.

The results for texture analysis at time zero for the whole potatoes identified a product with an important firmness that contributes to greater resistance to the mechanical damages during its postharvest handling, which could be attributed to the important pectic compounds present in the cell walls of the potato peels' tissue (Bordoloi, 2012). The penetration strength values found in the potato variety Primavera were higher than those reported by Castro Lara (2008), in fresh potatoes from the Hermes variety, stored at 4 °C, but lower than those reported by Espinosa *et al.* (1998).

The texture of the sliced potato identified in greater detail the level of deformation of the internal matrix, showing an important elastic component (slope F/D<45°). This result suggests that the potato's interior may be less turgid and firm (Singh *et al*., 2016). This last situation may be due to the plasticizing effect of water present in the matrix (Salvador, 2009). For the sliced potato, the behavior was consistent with the loss of water that the tuber suffered during the first 15 d, which makes it less elastic., and is also related to changes in starch content, which is degraded to simple sugars, making the sliced potatoes tend to be fractured (van Dijk *et al*., 2002a).

The values of penetration strength in potato's slices were lower than those found by García-Segovia *et al.* (2008)

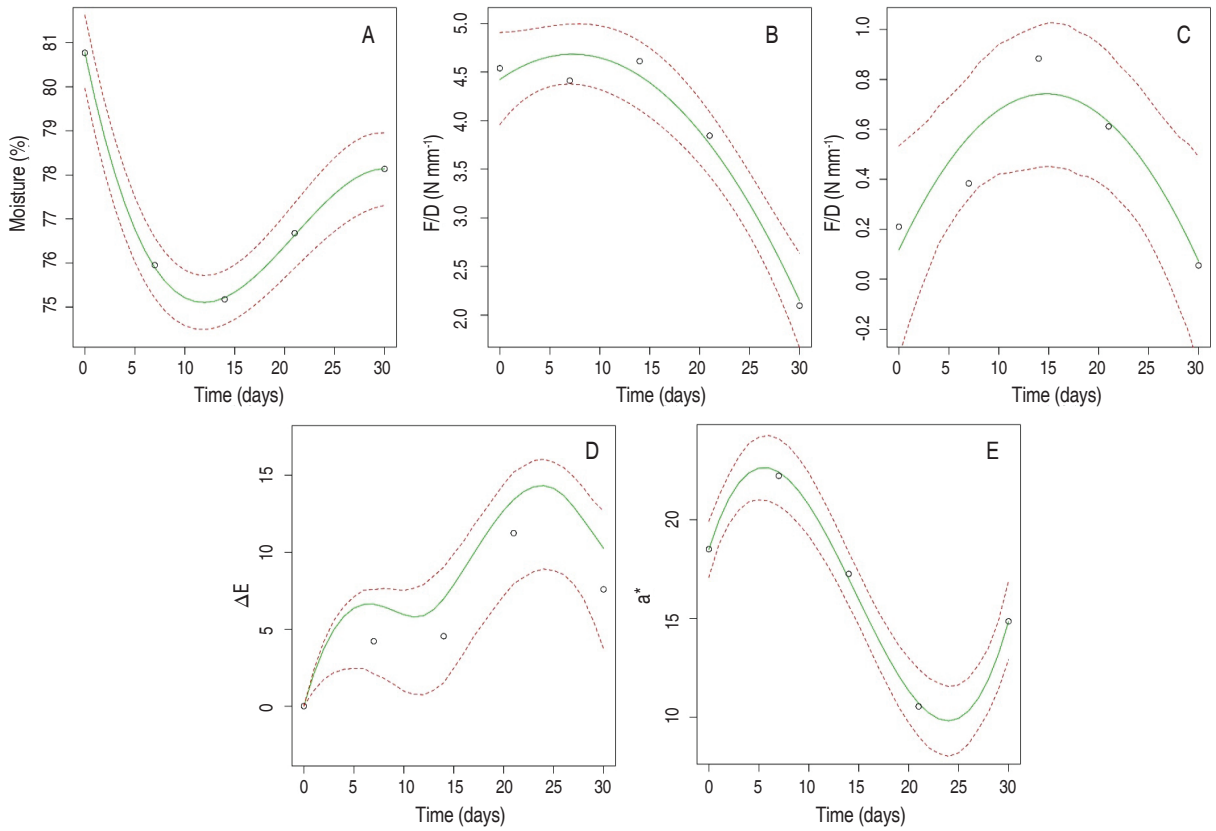

Figure 2. Mean values (o) and predicted values (green line) by the polynomial model for changes in potato physical attributes during storage time. A. Moisture; B. Texture for the whole tuber; C. Texture for slices; D. ΔE (color inside slice); E. a* (red-green chromaticity). Dashed red line shows both lower and upper boundary of the highest posterior density interval at 95% of probability.

for Monalisa variety of 20 mm diameter (397±35 N); therefore, it will be expected a less turgid, more elastic and collapsed structure product.

Changes in texture and moisture influenced the appearance of the tuber. From 14 d the tuber began to be rougher and brightness, this situation increased along the storage time (Figure 3).

The color change was adjusted to a cubic model. Figure 2 shows a gradual increase of ΔE in periods of 7 d, reaching its maximum value of 12.3 in 21 d, followed by a decrease of ΔE until 30 d.

The changes for ΔE are attributed mainly to three phenomena: initially to the sowing, geographical and environmental conditions that provide in potatoes a high variability between lots, in terms of size, pigmentation, and shape. On the other hand, and consistent with the above, to the greater or lesser density of pigments that the equipment captures in the observation window used in the spectrophotometer (ϕ=11 mm), which is random and uncontrollable. Finally, to the changes mainly in chromaticity a* attributable to the degradation of anthocyanins and carotenoid pigments present (Šulc *et al.*, 2017). The ΔE property showed an increase at the end of storage concerning 0 d of storage; this result is in agreement with the result obtained by Nourian *et al.* (2003) who found an increase in the color of the pulp for the Chieftain Potato variety, stored at different temperatures including 4°C.

The changes in pulp potatoes color during the storage should be due to chemical reactions, in the case of brightness, this change is usually seen during the transformation of amyloplasts to chloroplasts (Grunenfelder *et al.*, 2006), because of the light presence during storage. In the case of chromaticity a*, its variation is mainly changes suffered in colored compounds, like anthocyanins. These reddish

compounds are transformed in other types of compounds, by hydroxylation, methylation, and glycosylation; which generate color changes, by pH effects, temperature, and light (Reyes and Cisneros, 2007).

Figure 3. Photographs of potato cv Primavera during storage. A. 0 d; B. 7 d; C. 14 d; D. 21 d; E. 30 d.

The fit to the model for ABTS, DPPH and phenolic compounds (PC) was cubic (Figure 4). In the case of DPPH, there was a decrease in values up to 14 d (from 2.33 to 1.20 mg Trolox g^{-1}) followed by a slight increase (from 1.28 to 1.48 mg Trolox g^{-1}) (Figure 4B). Overall, a decrease of 50% in the antioxidant activity is observed by the DPPH method, and a decrease and subsequent increase in this quality attribute is observed with ABTS method (from 2.89 mg Trolox g^{-1} at 0 d to 2.94 mg Trolox g^{-1} at 30 d) and PC (6.09 mg GAE g^{-1} at 0 d to 6.27 mg GAE g^{-1} at the end of the storage).

The behavior of the parameters associated with the antioxidant capacity of the diploid potato (ABTS, DPPH, total phenols) did not show a specific correlation with the color parameters, probably due to the same variability mentioned for the ΔE. However, there are other factors not controlled in the research that may be affecting, such as those associated with primary production and the chemical and physical phenomena of the interaction of the matter with the environment.

The differences in both measures, ABTS and DPPH, occur because of the type of antioxidant assessed with each method. In general, ABTS measures hydro- and lipophilic compounds (Kuskoski *et al.*, 2005). DPPH measures hydrophobic antioxidant systems; Floegel *et al.* (2011) found great differences between both methods in highly pigmented foods, which is the case of potato variety Primavera. Similar behavior for antioxidant capacity is observed for colored potatoes, especially clone 'CO97227-2P/P', reported by Külen *et al.* (2013), in which antioxidant activity by ABTS showed a considerable increase during storage at refrigeration conditions for 7 months; in contrast, with DPPH, the antioxidant capacity was constant over time.

DPPH and ABTS values, at time 0 d, were higher than the one reported by Molina *et al.* (2015) (DPPH: 458 µmol Trolox 100 g^{-1} for clon 2 of native potatoes), and the ones reported by Burlingame *et al.* (2009) for *S. pinnatisectum* wild Andean species (43-892 µg Trolox g^{-1}).

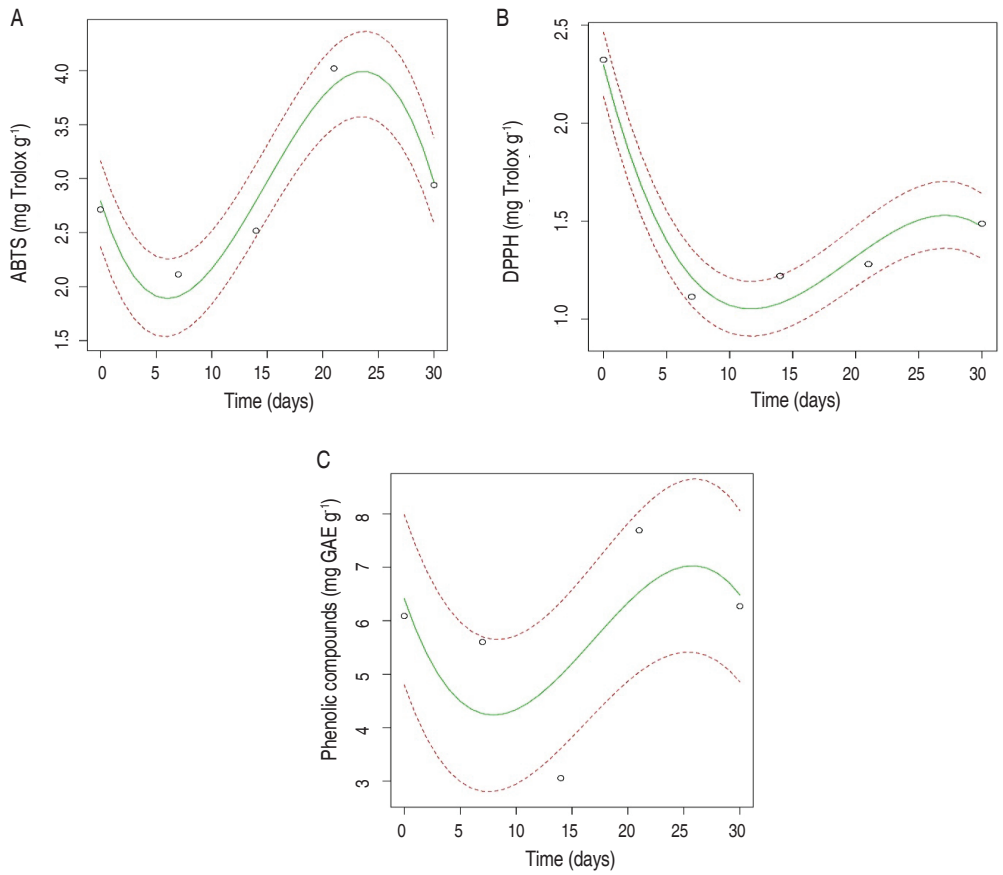

Figure 4. Mean values (o) and predicted values by the polynomial model in potato chemical attributes during storage time. A. ABTS; B. DPPH; C. Total phenolic compounds. Dashed red line shows both lower and upper boundary of the highest posterior density interval at 95% of probability.

An outstanding feature of this potato variety is its number of antioxidants, as well as phenolic compounds. Figure 4 presents a behavior similar to that one reported by Singh and Kaur (2016), which shows an increase in a number of total phenolic compounds, specifically the p-cumaric acid and quercetin, during potato storage, especially at temperatures of 4°C. Low temperatures, light, and some pathogens during storage could induce the generation of phenolic compounds, by the phenylalanine ammonia lyase, that regulates the synthesis of these compounds –considering that the majority of phenolic compounds in reddish diploid potatoes are anthocyanins (Rytel *et al*., 2014). The increase in total phenolic compounds with time in refrigeration conditions can be explained partly by enzyme anthocyanin synthase activity, which increases in lower temperatures (Dios-López *et al*., 2011) the degradation of the complex phenolic structures into phenolic acids, and de breakdown of cell structure which have the phenolic compounds strongly linked (Türkben *et al*., 2010).

CONCLUSIONS

Potato variety Primavera has antioxidant properties and total phenolic content higher than the found in other potatoes with similar features, making it food with great potential for industrial exploitation. The stability study allowed the evaluation of some properties of the potato during the storage time. A well-defined tendency of antioxidant activity and total phenolic compounds was not observed. At 21 d, the potatoes displayed considerable rugosity and loss of turgor, along with color deterioration, making the product a perishable food that requires fast consumption and processing after harvest. In the models evaluated, none showed a lack of fit.

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Effect of pruning, fertilization and pesticide injection on crown dieback in urban trees in Colombia: Analysis of factors involved

Efecto de la poda, fertilización e inyección de pesticidas sobre la muerte descendente en árboles urbanos en Colombia: Análisis de los factores involucrados

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ABSTRACT

This research evaluated the effect of pruning, fertilization and pesticide injection on crown dieback in urban trees in Colombia and analyzed the factors involved. Systemic insecticides and/or fungicides were applied through injections in the trunk of 15 tree species affected by the progressive deterioration of the crown in the urban forests of the Metropolitan Area of the Aburrá Valley. The presence of progressive deterioration was evaluated qualitatively on a scale from zero to three in an average sample of 12 individuals for each species. Two treatments were used: i) application of insecticide + fungicide, and ii) application of insecticide, both treatments were applied three times, plus a general treatment of pruning and fertilization. Also, two controls were evaluated: healthy trees and diseased trees without treatment. The probability of individual progressive deterioration (PD) during the study period according to the species, treatment applied, and the initial state of affectation was estimated using a generalized linear mixed model. The analysis of factors involved also included planting site, traffic flow of the site, the wood density of the species, and time. The results suggest that the deterioration is a dynamic phenomenon associated with environmental stresses caused mainly by the climatic variability. From the evaluated variables, the species seems to be the most determinant factor for the affectation, since intrinsic variables of the species, like wood density, can favor its appearance. On the contrary, the treatments evaluated did not affect the recovery of the species. The action of insects and pathogens seems to be opportunistic once trees are affected.

Keywords:
Fungicides
Insecticides
Progressive deterioration
Statistic modeling
Tree health
Urban forest

RESUMEN

Esta investigación evaluó el efecto de la poda, fertilización e inyección de pesticidas sobre la muerte descendente en la copa de árboles urbanos de Colombia y analizó los factores involucrados. Se aplicaron insecticidas y/o fungicidas sistémicos a través de inyecciones en el fuste sobre 15 especies arbóreas que han venido siendo afectadas por el deterioro progresivo de la copa en los árboles urbanos del Área Metropolitana del Valle de Aburra. Se evaluó de manera cualitativa la presencia de deterioro progresivo en una escala de 0 a 3, en una muestra de 12 individuos en promedio por cada especie. Se emplearon dos tratamientos: i) insecticida + fungicida; y ii) insecticida, aplicados en tres ocasiones, más un tratamiento general de poda y fertilización. También se evaluaron dos controles: árboles sanos y enfermos sin tratamiento. Mediante el uso de un modelo mixto lineal generalizado se determinó la probabilidad de presentar deterioro progresivo (DP) por individuo durante el periodo de estudio en función de la especie, el tratamiento aplicado y el estado inicial de afectación. El análisis de los factores involucrados también incluyó el sitio de plantación, el flujo de tráfico del sitio, la densidad de la madera, y el tiempo. Los resultados sugieren que el deterioro es un fenómeno dinámico asociado a tensores ambientales causados principalmente por la variabilidad climática. De las variables evaluadas, la especie parece ser el factor más determinante para la afectación, ya que variables intrínsecas de la especie, como la densidad de la madera, pueden favorecer su aparición. Por el contrario, los tratamientos evaluados no tuvieron ningún efecto sobre la recuperación de las especies. La acción de los insectos y patógenos parece ser oportunista una vez los árboles están afectados.

Palabras clave:
Fungicidas
Insecticidas
Deterioro progresivo
Modelado estadístico
Sanidad del árbol
Bosques urbanos

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In the urban environment; soils, microclimate, and other environmental conditions are often hostile to trees. Many urban microhabitats are not favorable for their growth and development, which frequently produces damage to the crown, branches, bark, and roots. Factors such as soil impermeability, hardness, and chemical composition, low nutrient and water inputs, the effect of contamination (high emissions of SO_2, NO_x, PM 2.5, PM 10, acid rain, among others), and thermal stress induced by urban heat islands cause physiological imbalance, by increasing the susceptibility to the attack of pests and diseases, and finally the tree's death (Jochner and Menzel, 2015).

Because of the restrictions imposed by the urban environment, many species do not survive or grow well in cities; features such as reduced planting space, high tree costs, low availability of planting material in local nurseries, and current societal needs –e.g., trees without allergenic pollen– reduce the number of potential species to be used in future plantations. The stress of trees in the urban environment affects their populations in two ways: firstly, the increase in mortality, which raises the need for subsequent removals and replacements; and secondly, and perhaps more important, the removal and replacement of trees tend to generate an oversimplification of the urban ecosystem (Conway and Vander Vecht, 2015).

From the management perspective, the main negative effect of the dominance of one or a few species in urban forests is the increased probability of death due to outbreaks and attacks of pathogens and insects, which may result in the devastation of whole areas in one single event (Tomlinson *et al*., 2015). Therefore, species diversity has been considered a good alternative to increase the resilience of the urban forests against the attacks of insects and diseases. However, a high diversity of species brings difficulties of management since it supposes a greater range of requirements and potential phytosanitary problems (e.g., pruning time, nutritional demands, pests, and diseases).

The health of urban trees is essential for them to provide the desired ecological and economic benefits, which is one of the most significant challenges for managers of the urban forest. It has been found that the application of systemic insecticides and fungicides on trees affected by phytosanitary problems of subtropical species (elm, ash, among others) generates benefits in the short and medium term (Dal Maso *et al*., 2014; Mercader *et al*., 2015). Likewise, preventive actions such as sanitary pruning and fertilization are important for the management of trees since they reduce the stress generated by the urban environment (Fini *et al*., 2015).

Among the most common active components in insecticides used for phytosanitary management of urban trees are imidacloprid, bifenthrin, and thiacloprid, as they act selectively on the central nervous system of insects and present a low risk for non-target organisms such as mammals (Poland *et al*., 2006; Jeschke and Nauen, 2008). Although these substances have been applied in different parts of the tree, the application in the stem through injections has become one of the most used methods because it generates the minimum risk of exposure and release of their active components to the environment. The insecticide applied in the injection is transported to the insect feed site through the xylem sap (Mota-Sanchez *et al*., 2009).

Few studies have focused on tree management and health in Latin American cities (Escobedo *et al*., 2006 in Chile; Tavares *et al*., 2013 in Brazil; Restrepo *et al*., 2015 in Colombia). These studies have evidenced a deteriorating trend in the urban forests of Latin America associated with the growing population and the expansion activities derived (i.e., Industrial agglomerations, slum growth, increased automobile fleet), as well as pests and diseases that attack plant species when they are under persistent stress situations such as those prevailing in the urban environment. However, few studies have focused on the phytosanitary diagnosis and management strategies of urban trees in Latin America (Sepúlveda Cano *et al*., 2009; Rodrigues *et al*., 2014; Zaragoza *et al*., 2014), so the knowledge about optimal protection and remediation measures for urban forests in the cities of this part of the planet is limited.

The urban trees in the Metropolitan Area of the Aburrá Valley (Colombia) have been presenting phytosanitary problems and symptoms of deterioration and dieback for at least the last decade. The affected individuals have shown the presence of several species of insects and fungi, which could be responsible; however, no systematic

search for the causal agents of this phenomenon has been done yet. As the first step in this search, a field experiment was performed to evaluate the effect of applying systemic insecticides and fungicides on the health of the crown of 15 tree species. The hypothesis is that the deterioration of tree crowns is generated by the action of insects and fungi, which is favored by the stress of trees in the urban environment. The specific objectives of the study are 1) to determine the effectiveness of treatments to reverse the symptoms associated with the deterioration of the aerial part of the treated individuals; 2) to identify some factors that could affect the temporal behavior of the individuals evaluated (during the experimental period, 2013 - 2015). It is expected that the results obtained will contribute to improving the knowledge of the management and health of trees in tropical cities.

MATERIALS AND METHODS

Study area

This study was carried out in the urban area of four municipalities (Itagüí, Medellín, Sabaneta, and Envigado), belonging to the Metropolitan Area of the Aburrá Valley, the urban center with the second largest concentration of population in Colombia, with an urban area of 345.6 km^2. The altitude varies between 1400 and 1600 m.a.s.l., with an average annual temperature between 22 and 25 °C, and an average rainfall of 2500 mm year^{-1} (AMVA and CONCOL, 2007). According to data collected from the weather station Olaya Herrera Airport during the experimental period (2013-2015), the rainfall is bimodal, with two humid periods in April-May and October-November, when the lowest average monthly temperatures occurred; and two dry periods (in June-July and December-January), with the highest temperatures (Figure 1).

Definition of the study variable and species selection

The variable of interest in this study was called progressive deterioration (PD), a phenomenon associated with symptoms of sudden death, wilting, branch rot, and downward drying in trees of the urban areas of the Aburrá Valley (Restrepo *et al.*, 2015). Symptoms start with the drying of upper branches, continuing with the intermediate branches, and ending with general deterioration and the death of the tree. The evaluation methodology consisted of recording the absence or presence of PD in three levels of severity: low (less than 20% of the crown affected), medium (affectation between 20 and 60% of the crown), and high (more than 60% of the crown affected).

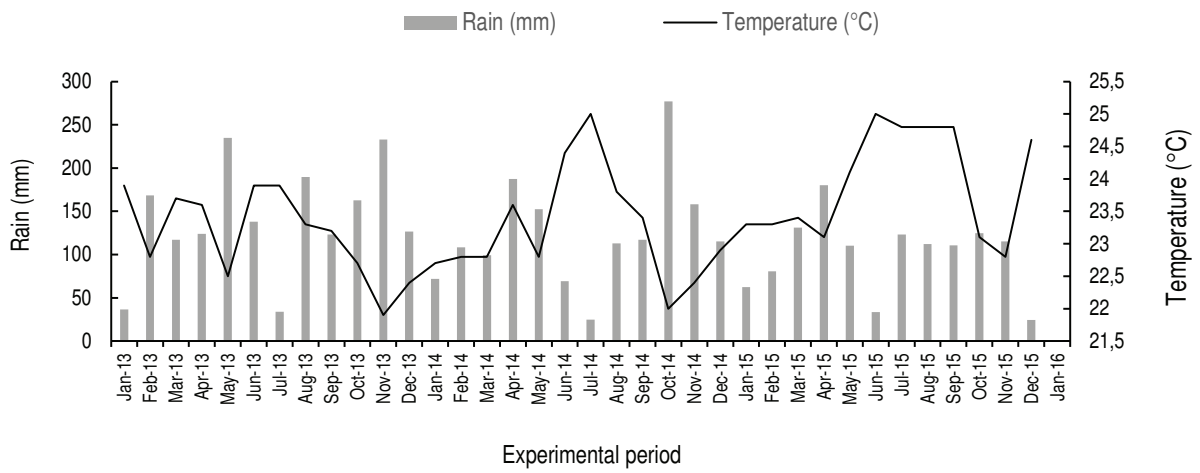

Figure 1. Monthly precipitation (mm) and average temperature (°C) at Olaya Herrera Station 2013 - 2015. (IDEAM, 2015).

It has been found that some species are more susceptible to exhibit the PD symptoms (Restrepo *et al.*, 2015; UNAL and AMVA, 2015); for this study 15 of them were selected, which also were abundant and widely distributed in the area (Table 1). It was evaluated the effect of a combined treatment, consisting of the application of systemic insecticides and fungicides plus a general treatment of sanitary pruning and fertilization as phytosanitary protection measures to reverse the PD affectation.

Table 1. List of species studied, value of incidence of progressive deterioration (PD) in years 2012 and 2014 (Restrepo *et al*., 2015; UNAL and AMVA, 2015, respectively), and wood density.

Species	Incidence PD 2012	Incidence PD 2014	Wood density	
			kg m^{-3}	Class
Archontophoenix cunninghamiana (H.Wendl.) H.Wendl. & Drude	0.001	0.03	750	Medium
Bauhinia picta (Kunth) DC.	0.12	0.16	640	Medium
Caesalpinia pluviosa DC.	0.06	0.11	890	High
Eriobotrya japonica (Thunb.) Lindl.	0	0.02	880	High
Ficus benjamina L.	0.19	0.17	459	Low
Fraxinus chinensis Roxb.	0.01	0.18	560	Medium
Handroanthus chrysanthus (Jacq.) S.O.Grose	0.05	0.12	920	High
Jacaranda mimosifolia D.Don	0.03	0.12	470	Low
Lafoensia punicifolia DC.	0.12	0.14	690	Medium
Lagerstroemia speciosa (L.) Pers.	0.05	0.13	530	Medium
Pithecellobium dulce (Roxb.) Benth.	0.17	0.16	517	Medium
Roystonea regia (Kunth) O.F.Cook	0	0.02	556	Medium
Spathodea campanulata P.Beauv.	0.09	0.21	250	Low
Terminalia catappa L.	0.01	0.06	562	Medium
Syzygium malaccense (L.) Merr. & L.M.Perry	0.06	0.17	483	Low

The treatments evaluated were (Table 2):

1. Negative control: healthy trees, which did not receive any treatment.
2. Positive control: trees affected by PD that did not receive any treatment.
3. Treatment 1 (T1): trees affected by PD and free of soil pathogens. This group received the application of systemic insecticide and beneficial fungi. The treatment consisted in the application in the base of the tree (in four equidistant points) of an intracambium injection of 20 cm^3 of systemic insecticide (Bifenthrin (50 g L^{-1}) + Imidacloprid (250 g L^{-1})) and the application to the soil of 20 g of a mixture of antagonistic fungi (*Trichoderma harzianum*, *T. koningii*, and *T. viridae*) dissolved in 10 L of water, as a preventive measure to inhibit the growth and development of soil phytopathogenic fungi.
4. Treatment 2 (T2): trees affected with PD and by soil pathogenic fungi. This group received the application of systemic insecticide and fungicide. The treatment consisted of the application in the base of the tree (on four equidistant points) of an intracambium injection of 20 cm^3 of systemic insecticide (Bifenthrin (50 g L^{-1}) + Imidacloprid (250 g L^{-1})) plus 20 cm^3 of a systemic fungicide (Carbendazim 500 g L^{-1}) dissolved in water to a concentration of 0.6 cm^3 L^{-1}.

To enhance the effect of treatments, individuals from T1 and T2 received three sanitary pruning of affected branches (with 50% or more of their length without leaves and with evidence of decay) and three soil fertilization (circular at 50 cm from the tree base) with a mixture of 400 g of triple 15 (N: 15%, P: 15%, K: 15%) + 100 g of a fertilizer with major and minor nutrients (N: 8%, P: 5%, Ca: 18%, Mg: 6%, S: 1.6%, B: 1%, Cu: 0.75%, Mo: 0.005%, and Zn: 2.5%).

A total of 188 adult individuals from 15 species were sampled, which were evaluated five times including the baseline measurement. Treatments along with pruning and fertilization were applied three times, in the baseline (July - August 2013), in the second (October - November 2013) and fifth measurement (March - April 2015).

Table 2. Number of individuals evaluated per species for each treatment and control.

Species	Number of individuals evaluated			
	T1	T2	Positive control	Negative control
A. cunninghamiana	4	4	3	3
B. picta	3	3	3	3
C. pluviosa	3	3	3	3
E. japonica	3	3	2	4
F. benjamina	3	3	3	3
F. chinensis	3	3	3	3
H. chrysanthus	3	3	3	3
J. mimosifolia	3	3	3	3
L. punicifolia	3	3	3	3
L. speciosa	3	3	3	3
P. dulce	3	3	3	3
R. regia	4	3	4	3
S. campanulata	3	3	3	3
T. catappa	3	3	3	3
S. malaccense	3	7	3	3
Total general	47	50	45	46

Statistical analysis

Contingency tables were built to evaluate the possible relationships between the categorical variables and the recovery of individuals between the baseline and the fifth measurement. The recovery was defined using a binary categorical variable, with 1 for recovery and 0 for no recovery of the individual; that is, if the individual went from any category of affectation (i.e., low, medium or high) to the absence of PD, this variable was equal to one.

Given that the nature of this study is longitudinal, and therefore the response has both a contribution of repeated measures per individual and a non-continuous response (binomial in this case), the construction of models to explain the presence of PD during the evaluation time was performed using generalized linear mixed models (GLMM) (Fitzmaurice *et al*., 2011). The model was based on the explanatory approach outlined by Schwartz and Lellouch (2009), and its purpose was to define the effect of how the tree was treated during the experimental period considering the variations that might occur (i.e., incomplete application of the treatment). The so-called full cases methodology was used for handling of dropouts, given the low percentage of dead individuals and/or cut trees. The model was fitted using the PROC GLIMMIX of the SAS® V9.4 software.

The adjusted generalized mixed model was:

$$Y_{ij}\left|bi \sim B(\pi_{ij}), \log\left\{\frac{pr(Y_{ij}=1)}{pr(Y_{ij}=0)}\right\} = \beta_0 + b_i + \beta_1 t_{ij} + \beta_2 sp_i + \beta_3 ISS_{i,j=1} + \beta_4 T1_{ij} + \beta_5 T2_{ij} + \beta_6 Fi_j\right.$$

Where Y_{ij} was the response variable, presence of PD measured in the *i*-th subject at time *j; i*=1,2,..., *N* denotes the subjects and *j*=1,2,..., n_i denotes the observations for the *i*-th subject at time *j* (longitudinal observations). Y_{ij} is a binary response taking the value of 1 when a specific tree shows PD (any of the three categories above mentioned), and 0 for no presence of PD. In both models, the link function to connect the linear predictor and the response was the logit. b_0 corresponds to a random intercept which was specified for each subject under study. By doing so, it is believed that the model can capture extra variability that a model based only on

fixed effects cannot capture. The vector of parameters $\beta=(\beta_1, \beta_2,..., \beta_6)$, includes the regression coefficients of the fixed effects of the model; β_0 is the population intercept of the model. Among the fixed effects, t_{ij} is the time of evaluation of the subject *i* at time *j* and was recorded as follows:

Baseline or first time: July-August 2013
Second time: October-November 2013
Third time: May-June 2014
Fourth time: March-April 2015
Fifth time: December 2015

sp_i corresponds to the *i*-th subject of the species (Table 1). $ISS_{i,j=1}$ is the initial severity state of PD of the *i*-th subject at the baseline. $T1_{ij}$ and $T2_{ij}$ correspond to the dichotomous variables Treatment 1 and Treatment 2 that were applied to the *i*-th subject (which takes values according to the time *j*), and F_{ij} is a dichotomous variable taking the value of 1 if fertilization on the *i*-th subject was applied at time *j*. This differentiation was done because it was not possible to apply treatments as planned in some individuals.

The so-called Residual Pseudo-Likelihood (RSPL) technique was used to obtain the estimates of both fixed and random effects. This technique uses a subject-specific expansion through the Taylor series. The variance-covariance matrix of fixed effects was obtained using the empirical estimator (sandwich estimator) to obtain a robust analysis taking into account the lack of specification of the covariance structure. The structure called *unstructured* was selected to calculate the variance-covariance matrix of the random effects. Paired comparisons between the levels of the variables that were significant in the models were performed. In all comparisons, $P<0.05$ was considered statistically significant.

The model building had several stages. At each stage the inclusion of variables was evaluated; among such variables are planting site (hard floor, tree-grate, and green zone), traffic flow of the site (high, medium and low), and dasometric variables of trees (diameter and height). However, none of these variables were significant and for that reason were not included in the model presented above.

Finally, the estimated probability of PD at the five evaluation times for each species was related to the wood density of the species (kg m^{-3}). Also, with the individual probability of suffering PD, profile plots per species were built to evaluate the trend of PD over time.

RESULTS AND DISCUSSION

From the 188 individuals evaluated, 13 had incomplete responses because death and cut (eight and five, respectively) and therefore they were not analyzed. Significant differences between species in terms of its recovery between the baseline and the fifth measurement (*Chi-squared*=34.21, df=14, $P<0.05$) were found. In contrast, recovery showed no significant difference neither between planting sites (*Chi-squared*=5.4959, df=2, $P>0.05$) nor the traffic flow of the site (*Chi-squared*=2.1185, df=2, $P>0.05$) (Table 3).

Table 3. Contingency table for the variables planting site and vehicular traffic. (1) There was recovery; (0) there was no recovery ($\alpha=0.05$). The Recovery columns show the number of individuals; the Comparison column shows the probability associated with the hypothesis test of equality of each comparison.

Variable		Recovery		Comparison	
		0	1		*P*
Planting site	Green zone	37	61	Green zone - Tree-grate	0.3454
	Tree-grate	11	10	Green zone – Hard floor	0.1155
	Hard floor	9	4	Tree-grate – Hard floor	0.4774
Vehicular traffic	High	24	35	High - Medium	0.4936
	Medium	24	23	High - Low	0.6374
	Low	9	17	Medium - Low	0.4936

The positive coefficients estimated in the model indicate that the respective level of the categorical variable increased the presence of PD, while a negative coefficient indicates a decrease in the probability of PD. In this way, it was found that the probability of PD decreased during the study period (*time*), being lower in the last measurement (Table 4), presenting a temporal pattern of decreasing, which it was found to be general. Thus, differences in the presence of PD at the evaluation times (*time*) were higher, and statistically significant, as they were more spaced in time, being the baseline and the 2nd measurement the ones that showed a greater difference in comparison to the other measurements. The last two measurements did not show differences concerning the probability of having PD (between 0.18 and 0.12, t=1.69, df=692, *P*>0.05) (Figure 2).

Table 4. * Estimation of fixed effects of the explanatory model. Standard errors in parentheses.

Variable	Level	Estimated		*P*	Significance
Intercept		2.0732	(1.2223)	0.0918	ns
Evaluation time	*1st*	4.9218	(1.0695)	<0.0001	***
	2nd	2.5575	(0.7098)	0.0003	
	3rd	1.0146	(0.3385)	0.0028	
	4th	0.4925	(0.3003)	0.1015	
	5th (RL)	0			
Species	*A. cunninghamiana*	-4.2471	(0.8688)	<0.0001	***
	B. picta	-0.5405	(0.8936)	0.5454	
	C. pluviosa	-3.8644	(0.9278)	<0.0001	
	E. japonica	-2.2331	(1.0793)	0.0389	
	F. benjamina	0.9221	(0.8836)	0.2970	
	F. chinensis	-3.2746	(1.0129)	0.0013	
	H. chrysantha	-2.4524	(1.0909)	0.0249	
	J. mimosifolia	-0.5260	(1.2182)	0.6660	
	L. punicifolia	-0.8698	(0.9958)	0.3827	
	L. speciosa	-1.2664	(1.2412)	0.3079	
	P. dulce	-1.9532	(1.0161)	0.0550	
	R. regia	-2.4980	(0.9074)	0.0061	
	S. campanulata	1.9070	(1.5862)	0.2297	
	S. malaccense	-4.3343	(0.8889)	<0.0001	
	T. catappa (RL)	0			
Treatment 1	*0: No*	0.3544	(0.3942)	0.3689	ns
	1: Yes (RL)	0			
Treatment 2	*0: No*	-0.2530	(0.4218)	0.5488	ns
	1: Yes (RL)	0			
Fertilization	*0: No*	-0.8140	(0.7967)	0.3073	ns
	1: Yes (RL)	0			
Initial state of severity	*0*	-8.3137	(1.6276)	<0.0001	***
	1	-0.0822	(0.6575)	0.9005	
	2	0.2844	(0.6668)	0.6698	
	3 (RL)	0			
-2ResLog-pseudolikelihood			59360.00		
Chi-squared/DF			0.86		

RL: reference level; ns: not significant; ***: significant

*PROC GLIMMIX SAS® 9.4 selects as RL the last level of the categorical variable and it is on which the significance of the differences is established; *P* value indicates the value of the probability of equality between categories versus RL; the significance column of the variable (s) indicates whether or not the variable significantly explains the probability of an individual having PD (α=0.05).

On the other hand, the presence of PD, as a function of the initial state of severity (ISS), was not significantly different when the individuals were truly affected (i.e., when they had a low, medium or high affectation at baseline); however, these levels showed differences with respect to the unaffected trees, called "negative controls" (Figure 2).

The applied treatments (treatment 1, and treatment 2; plus pruning and fertilization) did not show any influence at the individual level since trees treated showed the same behavior as those untreated (positive control) (Table 4). Contrary to these results, intracambium injections have been effective as a remedial and sanitary measure in urban trees of subtropical and temperate species such as *Ulmus* spp. (Poland *et al*., 2006), *Fraxinus* spp. (Mota-Sanchez *et al*., 2009), and *Tsuga* spp. (Doccola *et al*., 2007). However, these results probably are not comparable with this study, since there are marked differences with the tropical climatic conditions and species composition (both of the pathogen and the host) of the present investigation; besides, most of these studies assessed individual plague-host relationships, which facilitates dose evaluation, mechanisms of application, and effectiveness. Similar studies in this part of the tropics have not been reported (in terms of species and application of intracambium injections) as a remedial measure against the progressive deterioration of standing trees.

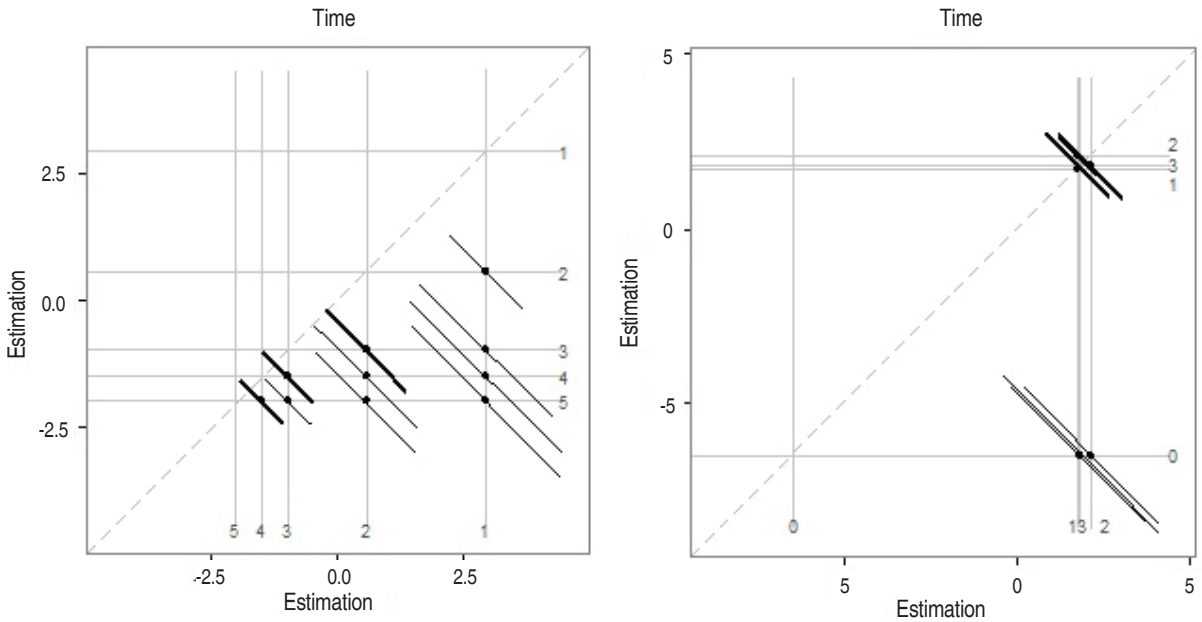

Figure 2. Least squares means (LSM) of the paired differences of evaluation times (left) and the initial state of severity (ISS, right) (α=0.05, Bonferroni adjustment). Dark lines: non-significant, clear lines: significant. The reference line (dotted line of 45°) shows a value of 0 for the confidence interval; as a consequence, comparisons whose confidence intervals cover zero through the reference line of 45 ° are not significant; on the other side, if the difference in the LSM is significant, the solid line for the confidence interval is completely above or below the dotted line of equality (High, 2015).

The applied treatments assumed that the deterioration of tree crowns is caused by insects and fungi, which in turn is favored by the stress of trees in the urban environment. However, these results did not support this hypothesis. An alternative explanation emerges from a close look at the climate data during this study. In the period of selection of the individuals studied (July - August 2013), the climate was dry and hot, the highest temperatures recorded for that year (Figure 1). Before this period, there was a wet season whose maximum rainfall occurred in May, with a drastic decrease of rainfall by almost 50% in June and an even more drastic decrease in July (34 mm precipitation in that month), which is atypical for the mild weather conditions of the region. This decrease in the availability of water probably produced stress in the individuals evaluated and consequently, the appearance of symptoms of crown deterioration. However, tree recovery occurred as precipitation increased and temperature declined in

subsequent months, suggesting a positive effect of increased water availability; which confirms the temporal pattern of PD. Therefore, although the attack of insects and fungi occurred, they appeared opportunistically, probably because of previous environmental stress; therefore, trees recovered as the stress diminished or disappeared.

Garrett *et al*. (2006) propose that there are climatic conditions that favor the pathogen and disfavor the host, which increases the potential for damage to trees. As a consequence, the pathogen-host relationship that occurs in some species of the Aburrá Valley probably obeys a seasonal pattern favored by the bimodal behavior of the climate in this zone, which causes stress in the trees during the times of greater radiation and temperature and lower precipitation, which benefits the proliferation of pathogens. Likewise, its harmful effect diminishes once the rains appear, when trees regain their vigor. This seasonal behavior of stressors would favor the existence of a physiological response called "acclimatization" (Niinemets, 2010), which would explain the recovery of most individuals, both treated and untreated.

At the species level, it was found that some of them were more susceptible than others. Species like *A. cunninghamiana*, *S. malaccense*, and *C. pluviosa* had a lower probability of occurrence of PD in contrast with *S. campanulata*, *F. benjamina*, and *T. catappa* (Table 4, Figure 3). Previous studies (Restrepo *et al*., 2015; UNAL and AMVA, 2015)

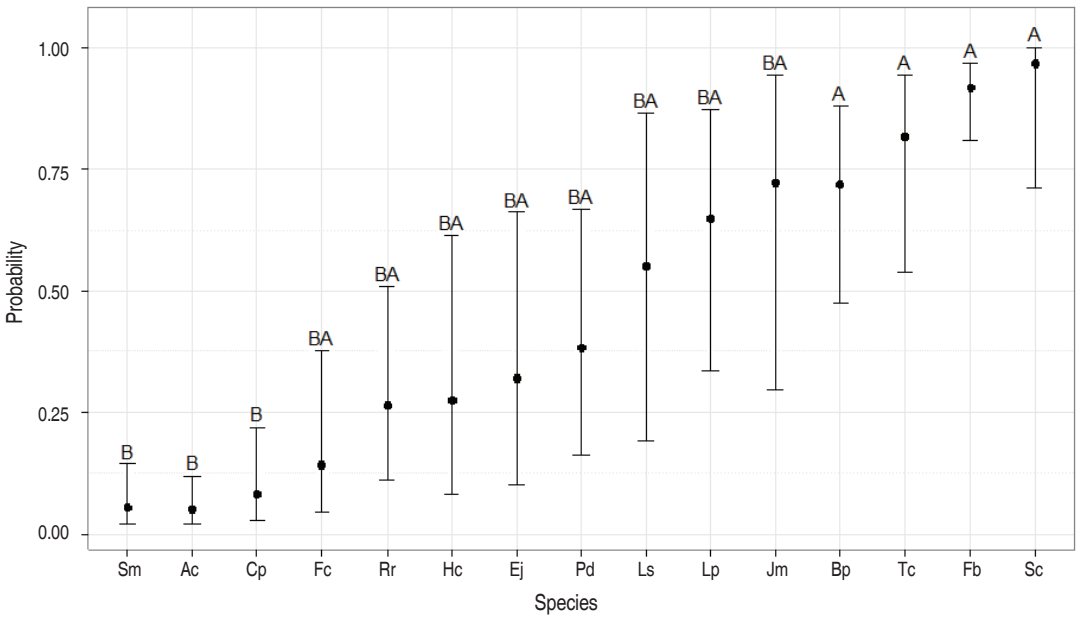

Figure 3. Confidence intervals of the probability of PD by species. Different letters indicate significant differences. Sm: *S. malaccense*, Ac: *A. cunninghamiana*, Cp: *C. pluviosa*, Fc: *F. chinensis*, Rr: *R. regia*, Hc: *H. chrysantha*, Ex: *E. japonica*, Pd: *P. dulce*, Ls: *L. speciosa*, Lp: *L. punicifolia*, Jm: *J. mimosifolia*, Bp: *B. picta*, Tc: *T. catappa*, Fb: *F. benjamina*, Sc: *S. campanulata*. Values with the same letter are not significantly different among them (ls-means PROC GLIMMIX SAS® 9.4).

for the same species in the study area reported a high correlation between the probability of PD occurrence and the incidence, confirming that the susceptibility to PD also depends on the species. Species such as *S. campanulata*, *F. benjamina*, and *T. catappa* have shown high susceptibility to the attack of sucking insects, borers, and defoliators in urban areas; which act as deleterious phytosanitary agents (Bito, 2007; Held and Boyd, 2008; Begoude *et al*., 2010; Arthurs *et al*., 2011; Maruyama *et al*., 2012; Tavares *et al*., 2013; Tavares *et al*., 2014; Herrera Isla *et al*., 2015; Lima *et al*., 2016) and explains in part the behavior shown by these species in this study.

The estimated probabilities of PD during the study period were different for the evaluated subjects (Figure 4). The individual profiles by species showed a general trend in

which a group of individuals maintained the very low probability of PD throughout the study; these were the subjects selected as positive controls, and their initial assessment of severity equaled to 0 (Figure 4). It was also noticed that subjects of *A. cunninghamiana*, *C. pluviosa*, and *S. malaccense*, affected at baseline, rapidly decreased the probability of PD and remained in that situation during the rest of the study. This trend was not observed in species such as *B. picta*, *F. benjamina*, *S. campanulate*, and *T. catappa*, whose probability of PD was high most of the time. Other species, such as *E. japonica*, *F. chinensis*, *H. chrysantha*, *J. mimosifolia*, *L. punicifolia*, *L. speciosa*, *P. dulce*, and *R. regia* did not show a clear trend in terms of the probability of suffering PD, and consequently, it was not possible to build a cluster with their profiles.

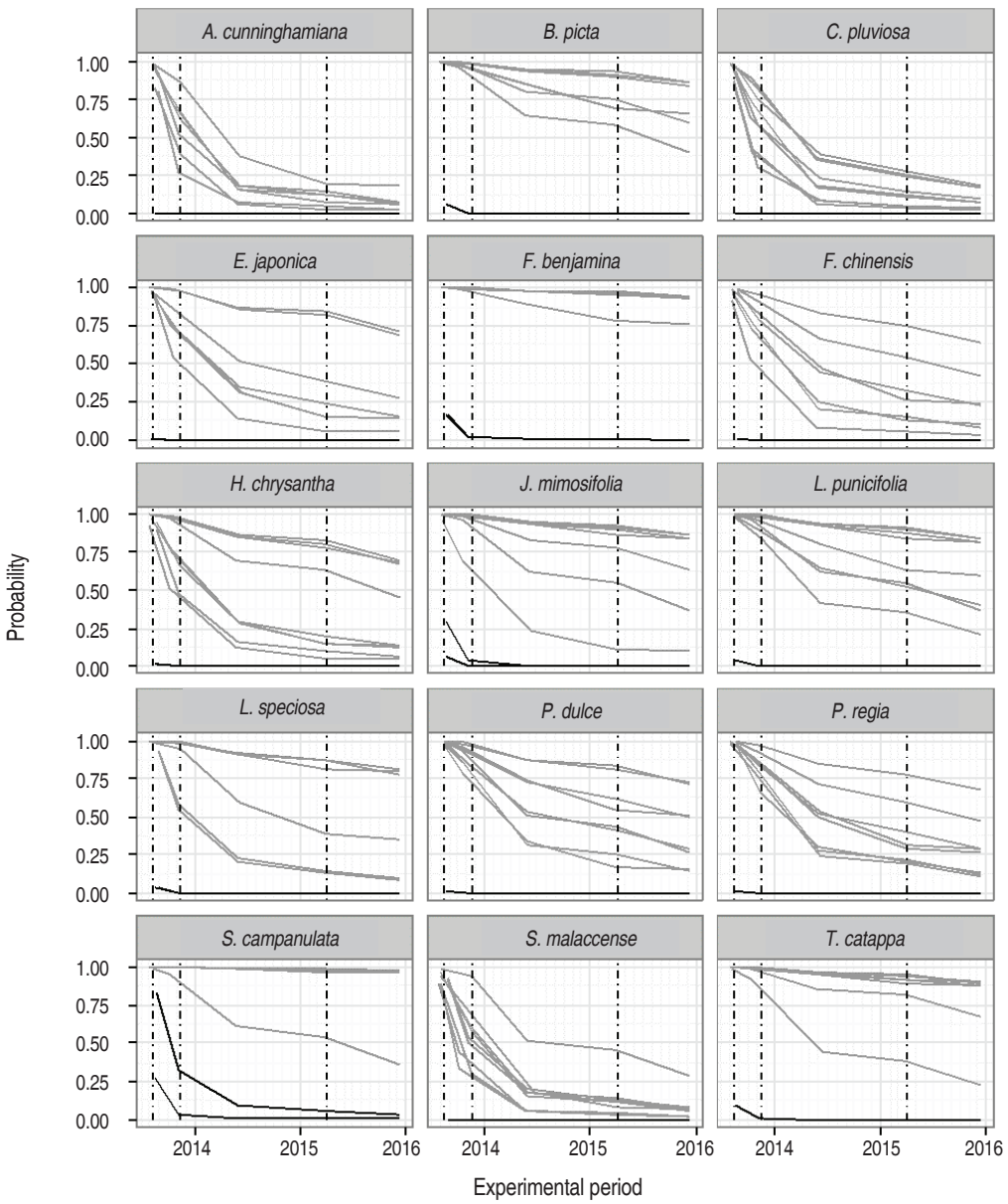

Figure 4. Individual probability profiles of PD during the study period by species according to the model. Vertical discontinuous lines show the times of treatment application. The color of the line indicates the initial state of affectation, gray: affected, black: healthy.

Tree crown health may be impaired by multiple causes, including water deficit, attack of insects, fungi, viruses, phytoplasmas, nutritional deficiencies (and the interaction among them); therefore, the diagnosis and treatment are complex, especially if one considers that different attacks may exhibit similar symptoms in different species. It has been reported that not only species do differ widely in their tolerance to different environmental factors (Philip and Azlin, 2005, Percival *et al.*, 2006), but also that the relation individual-species has a great effect on the longevity and survival of trees. Thus, a species can present individuals with high capacity for recovery (or acclimatization), as well as individuals that do not achieve a satisfactory recovery of their carbon stores after suffering successive stresses, causing irreversible physiological alterations (i.e., damage to photosystem II that inhibits photosynthesis, limited hydraulic conductivity, alteration in gas exchange, etc.), which may explain why some individuals did not show symptoms of recovery during the experimental period.

Finally, by species, it was observed that wood density showed a high correlation (nonlinear) with the probability of occurrence of PD (Spearman correlation coefficient=-0.70); in other words, such probability increased in species with low wood density. Likewise, the Kruskal-Wallis test showed that the classes of density were significantly different with respect to their probability of PD (P<0.05); thus, the pairwise comparisons of these classes showed that softwood species had significantly higher probability of having symptoms of PD (Figure 5) than those with medium and high density (P<0.05).

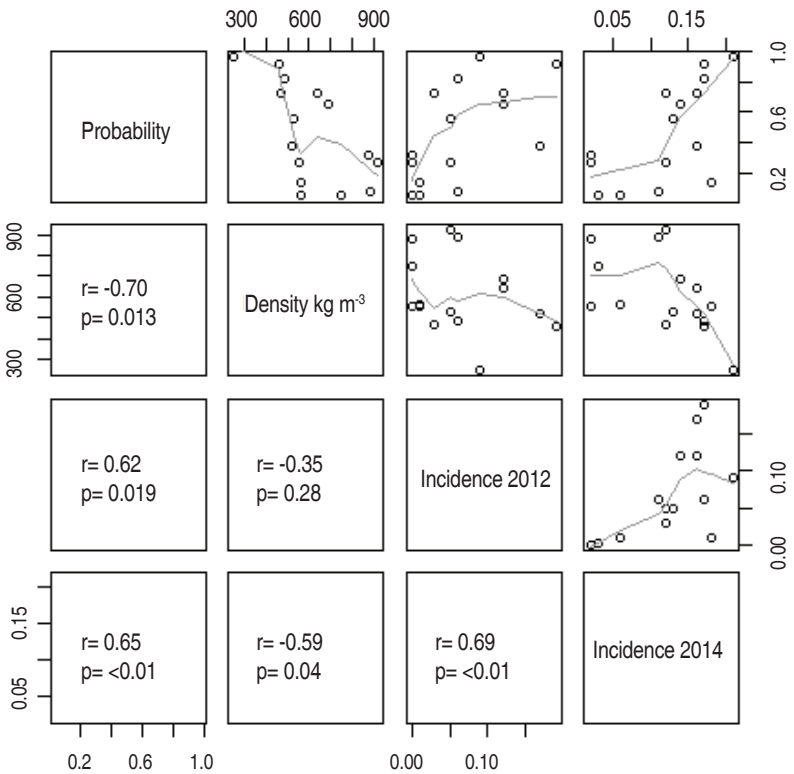

Figure 5. Matrix of non-linear correlation (Spearman) of the probability of PD (found in this study), wood density, the incidence in 2012 and incidence in 2014 per species.

It has been proposed that wood density is positively correlated with resistance to rot, since the high density may constitute a potential mechanism to reduce the risk of wounds (by falling branches) and the subsequent pathogen attack (Chave *et al.*, 2009). Larjavaara and Muller-Landau (2010) propose that high-density woods rot slowlier since there is less surface on which spores can land, and more solid structures between the bark

and pith, which increase resistance to penetration. In the same way Hacke *et al.* (2001), present that the size of the xylem vessels is related to wood density, as denser wood species generally have smaller vessels; this, in turn, leads to lower rates of water transport, but it represents greater resistance to xylem cavitation during droughts.

Individuals that remained unaffected throughout the study suggest high resistance to PD in most species analyzed. It is necessary to inquire about the physiological and genetic conditions that favor such phytosanitary status of this group of trees, which will help to improve the understanding of this phenomenon and its management. Also, the results suggest that effective treatments to maintain or improve urban tree health in subtropical areas are not necessarily applicable to the conditions of tropical cities.

CONCLUSION

The results obtained in the present study suggest that PD is a highly dynamic phenomenon in time that seems determined mainly by the climatic variability, which is facilitated by genetic characteristics of trees, such as wood density, and their physiological condition. The action of insects and pathogens seems to be opportunistic once trees are affected.

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POLÍTICA EDITORIAL

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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.

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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.

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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.

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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

Ponencias en memorias de congresos, seminarios, simposios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. pp. 65-94. En: Memorias IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo, Bogotá.

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Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae) (Tesis de maestría). Universidad Nacional de Colombia. Medellín, Colombia. 78 p.

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.

Taking into account Colciencias (Administrative Department of Science, Technology and Innovation of Colombia) criteria, the journal welcomes papers of the following types:

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Articles must be submitted in accordance with the guidelines set forth in “Instructions to Authors”; those who violate the rules will not initiate the basic editorial process. Shall be filled the form “Authorization for Release of Works and Economic Rights Assignment”, which will be provided by the Journal. This document is explicit in mentioning that all authors are informed and agree with article submitted for consideration to the Journal, that there is no conflict of interest between them, and also state that the manuscript has not been and will not be submitted for publication to another Journal.

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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.

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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).

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- 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)

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

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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.

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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.

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1.1. Los artículos deben contener suficiente detalle y referencias que permitan replicar o rebatir el estudio.

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2.6. Antes de iniciar la investigación se recomienda documentar la función y la forma como se reconocerá la autoría de cada investigador.

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4. Conflicto de intereses[4]

Criterios:

4.1. Cuando un investigador o autor, editor tenga alguna opinión o interés financiero/personal que pueda afectar su objetividad o influir de manera inapropiada en sus actos, existe un posible conflicto de intereses. Este tipo de conflictos pueden ser reales o potenciales.

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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".

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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.

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6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

7.1. El fraude en la publicación científica hace referencia a la presentación de datos o conclusiones falsas que no fueron generados a través de un proceso riguroso de investigación.
7.2. Existen los siguientes tipos de fraude en la publicación de resultados de investigación:
a) Fabricación de datos. Inventar datos y resultados de investigación para después comunicarlos.
b) Falsificación de datos. La manipulación de materiales de investigación, imágenes, datos, equipo o procesos.
La falsificación incluye la modificación u omisión de datos o resultados de tal forma que la investigación no se representa de manera precisa. Una persona podría falsificar datos para adecuarla al resultado final deseado de un estudio.

Recomendaciones:

7.3. Antes de enviar un artículo, lea cuidadosamente las políticas editoriales y de datos de la revista.
7.4. Nunca modifique, cambie u omita datos de forma intencional. Esto incluye materiales de investigación, procesos, equipos, tablas, citas y referencias bibliográficas.
7.5. Tanto la fabricación como la falsificación de datos son formas de conducta incorrecta graves porque ambas resultan en publicaciones científicas que no reflejan con precisión la verdad observada.
7.6. El autor debe hacer una gestión adecuada de los datos que soportan la investigación, teniendo especial cuidado en la recopilación, producción, conservación, análisis y comunicación de los datos.
7.7. Mantenga registros minuciosos de los datos en bruto, los cuales deberán ser accesibles en caso de que un editor los solicite incluso después de publicado el artículo.

8. Plagio[7]

Criterios:

8.1. El plagio es una de las formas más comunes de conducta incorrecta en las publicaciones, sucede cuando uno de los autores hace pasar como propio el trabajo de otros sin permiso, mención o reconocimiento. El plagio se presenta bajo formas diferentes, desde la copia literal hasta el parafraseado del trabajo de otra persona, incluyendo: datos, ideas, conceptos, palabras y frases.
8.2. El plagio tiene diferentes niveles de gravedad, como por ejemplo:
a) Qué cantidad del trabajo de otra persona se tomó (varias líneas, párrafos, páginas, todo el artículo)
b) Qué es lo que se copió (resultados, métodos o sección de introducción).
8.3. El plagio en todas sus formas constituye una conducta no ética editorial y es inaceptable.
8.4. La copia literal solo es aceptable si indica la fuente e incluye el texto copiado entre comillas.

Recomendaciones:

8.5. Recuerde siempre que es esencial reconocer el trabajo de otros (incluidos el trabajo de su asesor o su propio trabajo previo) como parte del proceso.
8.6. No reproduzca un trabajo palabra por palabra, en su totalidad o en parte, sin permiso y mención de la fuente original.
8.7. Mantenga un registro de las fuentes que utiliza al investigar y dónde las utilizó en su artículo.
8.8. Asegúrese de reconocer completamente y citar de forma adecuada la fuente original en su artículo.
8.9. Incluso cuando haga referencia a la fuente, evite utilizar el trabajo de otras personas palabra por palabra salvo que lo haga entre comillas.
8.10. El parafraseado solo es aceptable si indica correctamente la fuente y se asegura de no cambiar el significado de la intención de la fuente.
8.11. Incluya entre comillas y cite todo el contenido que haya tomado de una fuente publicada anteriormente, incluso si lo está diciendo con sus propias palabras.

9. Fragmentación[8]

Criterios:

9.1. La fragmentación consiste en dividir o segmentar un estudio grande en dos o más publicaciones.
9.2. Como norma general, con tal de que los "fragmentos" de un estudio dividido compartan las mismas hipótesis, población y métodos, no se considera una práctica aceptable.
9.3. El mismo "fragmento" no se debe publicar nunca másde una vez. El motivo es que la fragmentación puede dar lugar a una distorsión de la literatura haciendo creer equivocadamente a los lectores que los datos presentados en cada fragmento (es decir, artículo de revista) se derivan de una muestra de sujetos diferente. Esto no solamente sesga la "base de datos científica", sino que crea repetición que hace perder el tiempo de los editores y revisores, que deben ocuparse de cada trabajo por separado. Además, se infla injustamente el número de referencias donde aparece citado el autor.

Recomendaciones:

9.4. Evite dividir inapropiadamente los datos de un solo estudio en dos o más trabajos.
9.5. Cuando presente un trabajo, sea transparente. Envíe copias de los manuscritos estrechamente relacionados al manuscrito en

cuestión. Esto incluye manuscritos publicados, enviados recientemente o ya aceptados.

10. Consentimiento informado

Criterios:

10.1. Los estudios sobre pacientes o voluntarios requieren la aprobación de un comité de ética.

10.2. El consentimiento informado debe estar debidamente documentado.

10.3. Los permisos y las liberaciones deben ser obtenidos, cuando un autor desea incluir detalles de caso u otra información personal o imágenes de los pacientes y cualquier otra persona.

10.4. Especial cuidado debe tenerse con la obtención del consentimiento respecto a los niños (en particular cuando un niño tiene necesidades especiales o problemas de aprendizaje), donde aparece la cabeza o la cara de una persona, o cuando se hace referencia al nombre de un individuo u otros datos personales.

11. Corrección de artículos publicados[9]

Criterio:

Cuando un autor descubre un error o inexactitud significativa en el trabajo publicado, es obligación del autor notificar de inmediato a la revista y cooperar en el proceso de corrección.

Referencias

Black, William, Rodolfo Russo, y David Turton. «The Supergravity Fields for a D-Brane with a Travelling Wave from String Amplitudes». Physics Letters B 694, n.º 3 (noviembre de 2010): 246-51.

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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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[7] Elsevier, «Plagio. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0016/183400/ETHICS_ES_PLA01a_updatedURL.pdf.

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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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