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Periodicidad: Cuatrimestral
Vol. 70 No. 2 - 2017

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Dirección postal: Apartado Aéreo 568, Medellín, Colombia
Dirección electrónica: rfnagron_med@unal.edu.co
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Teléfono: (*4) 430 90 06; Fax: (* 4) 230 04 20
Diagramación: Miryam Ospina Ocampo
Marcación: LandSoft S.A.
Diseño e Impresión: Pato Amarillo Estudio de Diseño
Primera edición: Año 1939
ISSN: 0304-2847
ISSN formato web: 2248-7026
Doi: 10.15446/rfnam

Licencia Ministerio de Gobierno: 275/64

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Darwin Hernández Herrera. Facultad de Ciencias Agropecuarias. Universidad de Sucre. Sincelejo, Colombia - darwin.hernandez@unisucre.edu.co

Demian Takumasa Kondo Rodríguez. Corporación Colombiana de Investigación Agropecuaria - Corpoica Palmira, Colombia - takumasa.kondo@gmail.com

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Hernán González Osorio. Centro Nacional de Investigaciones de Café - Cenicafé. Chinchiná, Colombia. Hernan.Gonzalez@cafedecolombia.com

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Johana Andrea Ciro Galeano Fundación Universitaria Autónoma de Las Américas. Medellín, Colombia. johana.ciro@uam.edu.co

José Aires Ventura. Institute Capixaba de Pesquisa - INCAPER. Vitória, Espírito Santo, Brasil. ventura@incaper.es.gov.br

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José Mauricio Montes Rodríguez. Instituto Colombiano Agropecuario - ICA. Cúcuta, Colombia - jose.montes@ica.gov.co

J**uan David Corrales Álvarez.** Facultad de Ciencias Agropecuarias, Universidad de La Salle. Bogotá, Colombia - jdcorrales@unisalle.edu.co

Mark P. Culik. Institute Capixaba de Pesquisa - INCAPER. Vitória, Espírito Santo, Brasil. markculik@hotmail.com

Orelvis Portal Villafaña. Facultad de Ciencias Agropecuarias. Universidad Central "Marta Abreu" de Las Villas. Villa Clara, Cuba - orelvis@ibp.co.cu

Orlando Ruiz Villadiego. Facultad de Ciencias. Universidad Nacional de Colombia, Sede Medellín. Medellín, Colombia - osruiz@unal.edu.co

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Raúl Zapata. Facultad de Ciencias. Universidad Nacional de Colombia, Sede Medellín. Medellín, Colombia - rdzapata@unal.edu.co

Rita de Cássia Siqueira Bahia. Escola Politécnica da Universidade Federal da Bahia. Salvador - Bahia - Brasil - cassiabahia@yahoo.com.br

Santiago Ostengo. Estación Experimental Agroindustrial Obispo Colombres - EEAOC, Las Talitas, Argentina - santiagostengo@eeaoc.org.ar

Walter Osorio. Facultad de Ciencias. Universidad Nacional de Colombia, Sede Medellín. Medellín, Colombia - nwosorio@gmail.com

William Chavarriaga Montoya. Facultad de Ciencias Agropecuarias. Universidad de Caldas. Manizales, Colombia - william.chavarriaga@ucaldas.edu.co

Relación entre el efecto invernadero y el cambio climático desde la perspectiva del sector agrario

El objetivo de este editorial, es analizar el efecto invernadero y el cambio climático o calentamiento global atribuido, en parte, a las actividades desarrolladas o influenciadas por el hombre al avanzar en su proyecto de vida en el campo agrícola, pecuario, forestal y agroindustrial. Es necesario referirse a las faenas agrarias en donde se utiliza maquinaria agrícola, fertilizantes, insecticidas, fungicidas y en general al uso de agroquímicos. Así mismo, es necesario tener en consideración, la deforestación, las quemas de sabanas y bosques y el sistema alimentario del ganado, entre otras actividades agropecuarias y forestales. Es importante señalar, que las actividades humanas en general, vienen modificando, en parte, la composición de la atmósfera y el clima, lo que repercute, principalmente, en el ser humano, en la fauna, la flora, y que pone en riesgo, la seguridad alimentaria.

El efecto invernadero se conoce como la absorción que realiza la atmósfera de la radiación térmica emitida, por el sol, por la tierra y por los océanos, la cual es irradiada nuevamente hacia la tierra incrementando la temperatura de la superficie de la misma, proceso natural que permite que en la tierra exista vida. Para un mayor entendimiento en términos generales y sencillos lo que es el cambio climático o calentamiento global, se estableció un esbozo integrado por 9 puntos:

1. La geografía una ciencia necesaria. Es preciso visualizar, que la ciencia de la geografía, tiene una rama muy importante que se denomina geografía física. Por medio de ella, entre otras cosas, se puede conocer el paisaje de la tierra, su formación, el suelo, la agricultura, la ubicación de los ecosistemas y, la manera cómo actúa el clima sobre ellos. (Avilan J y Eder H. 1986)

2. La atmósfera. Es importante recordar, que la atmósfera se compone de la troposfera y la estratosfera. La troposfera es la capa más baja de la atmosfera que rodea a la tierra. Su espesor es de 8 km, en los polos y de 17 km, en el ecuador, sobre el nivel del mar. Su temperatura está alrededor de 20 °C. En esta capa se generan todos los fenómenos meteorológicos que afectan la naturaleza de la tierra, sean estos positivos o negativos. La estratosfera se localiza a 50 km sobre el nivel del mar, con una temperatura de 70 °C. En ella se ubica la capa de ozono (oxigeno trivalente), la que controla las radiaciones ultra violetas proveniente del sol y, a su vez, retiene los gases de efecto invernadero. En la atmosfera se generan gran parte de los fenómenos climáticos que actúan e influyen sobre el medio ambiente, especialmente, sobre los seres vivos que habitan la tierra.

3. La ecología y el medio ambiente. Para hablar de ecología y de medio ambiente, es preciso retrotraerse al año de 1869, cuando el zoólogo alemán Ernst Häeckel, promueve la creación de una ciencia, a la que denomino ecología. En ella, se establece una organización biológica, a la que llamó ecosistema. Para él, los ecosistemas estaban compuestos por seres vivos, que se relacionaban entre sí y, con el ambiente que los rodea. Fue la primera vez, que se mencionó la palabra ambiente. Por otra parte, es necesario indicar el significado de la palabra ecología; su significado proviene de la raíz griega OIKOS, que quiere decir, hogar, casa, patrimonio. Sumado a la ecología, apareció la Ciencia Ambiental, que nace posteriormente, en la década de los 60 del siglo XX. Ella establece como medio ambiente: todo lo que rodea a los seres vivos y los factores que influyen sobre ellos, como son: la luz, la temperatura, el agua, el suelo y el clima, entre otros. Ambas ciencias, estudian el patrimonio de la naturaleza, que está compuesto por los Ecosistemas.

4. Los ecosistemas naturales y artificiales. Se da el nombre de ecosistema a la comunidad de los seres vivos cuyos procesos vitales se relacionan entre sí y se desarrollan en función de los factores físicos de un mismo ambiente (RAE: Real Academia Española). En Colombia existen los ecosistemas naturales y los ecosistemas artificiales. Los primeros se encuentran en todas las regiones del país, desde los páramos, las laderas andinas, las selvas tropicales, los humedales, los llanos y los desiertos. Los ecosistemas artificiales o Agroecosistemas, son generadores de una gran cantidad de biomasa productora del gas metano, lo que influye negativamente sobre el Efecto Invernadero, al generar dióxido de carbono. Estos ecosistema están relacionados, con más de 60 cultivos que se siembran en el país, los cuales se reparten en, aproximadamente, 7,1 millones de ha. (Márquez CCJ. Revista Facultad Nacional de Agronomía Volumen 70 No. 1. 2007)

El territorio colombiano se encuentra en la zona ecuatorial, entre el trópico de cáncer y el trópico de capricornio; por tal motivo, posee una gran variedad de climas según la altitud de sus regiones naturales, lo que marca unas características diferenciales entre una y otra. El país es rico en ecosistemas naturales tropicales, lo que favorece el ambiente y, contribuye a mejorar el

efecto invernadero. La temperatura media anual, oscila entre 0 y 30 °C. Así mismo, las precipitaciones promedias anuales, puede variar de 400 mm en la Guajira, a 1000 mm en los Llanos Orientales, a 1500 mm en Medellín, a 1800 mm en el valle de San Nicolás, oriente antioqueño, a pluviometrías entre 2800 mm a 3000 mm en Bogotá y a 7500 mm en algunas zonas del Chocó. Colombia no posee, una estación de lluvia o invierno bien diferenciado, aunque abril y mayo, así como octubre y noviembre, tienden a ser los meses más lluviosos.

Por otra parte, la vegetación es muy variada, e incluye la selva tropical que se encuentra en la región amazónica, los humedales, los pantanos, los manglares del litoral Pacífico y del Atlántico, las sabanas de los llanos orientales, los matorrales de la Guajira y el bosque tropical de árboles bajos. Además, las praderas de las cuencas del Cauca y Magdalena y la vegetación de las zonas de nieves perpetuas.

Los gases, que generan el Efecto Invernadero (GEI), es un grupo minoritario de gases, que hacen parte de la atmosfera. Dentro de ellos se encuentran: el vapor de agua, el dióxido de carbono, el metano, el óxido nitroso, los perfluocarbonos, los hidrofluocarbonos y el exifloreno de azufre. Los tres últimos gases mencionados, son industriales. Los restantes, pueden ser naturales o, provienen de actividades humanas familiares. El IDEAM (Instituto de Hidrología, Meteorología y Estudios Ambientales de Colombia), considera, que el aumento de la temperatura en Colombia, en la próxima década, es real. Es posible, que la temperatura de algunas regiones aumente entre 2 y 4 °C, si esto ocurre, Bogotá podría alcanzar una temperatura similar a Girardot y las ciudades que tienen un clima cálido como Cartagena, serán invivibles. Colombia es víctima del Calentamiento Global.

5. El clima y el hombre. Clima, es el conjunto de elementos y factores atmosféricos y meteorológicos que caracterizan una región y, que determinan condiciones ecológicas propias. El clima es el elemento de la atmosfera que influye de forma determinante, tanto en los seres humanos, como en la fauna, flora y en los ecosistemas. La climatología, comenzó a tratarse como una ciencia en el siglo XIX. Por lo tanto, a continuación, se hará un breve listado, cronológico, de nombres de científicos, profesionales, técnicos y, de organizaciones de diferentes países, que han aportado sus conocimientos, para definir, caracterizar y clasificar el clima:

1882 Julius Hahm, Alemania; 1900-1936 Wilhelm Koppen, Alemania; 1934 Max Sorre; 1960 Enriqueta García; 1969 François Durand-Daste, Francia; 1970 P. Pedaborde; 1945-1978 Thornwaite, USA; 1981 FAO, Roma; 1982 César Mendoza de Armas ,Venezuela; 1994 Garduño; 1996 UNESCO-FAO, Francia, Roma; 2005 S. Mazparrote, Venezuela; 2005 Campos Aranda, México; 2007 WMO; 2009 Manuel López, México.

En conclusión, las actividades de la sociedad humana, sean estas industriales, agrícolas, vehiculares o de otra índole, han incidido, en la evolución del clima de zonas densamente pobladas, las cuales, ambientalmente, se conocen como, “Isla de Calor”. Muchos de los gases emitidos por las actividades mencionadas, se llaman gases de invernadero y ellos están generando el Efecto Invernadero Inducido (GEII), el que influye en el cambio climático. (Mendoza, 2009-2016b; Chémery, 2003; Hincapie y Mendoza, 1992).

El científico, Andy Javis, del CIAT (Centro Internacional de Agricultura Tropical, Cali Colombia), señala que la temperatura de Colombia ha aumentado 0,6 °C, lo que ha determinado, que el cultivo de café, deba sembrarse en un piso térmico de 1400 m y no de 1200 m de altitud, como se venía sembrando. Esto es apoyado por las asociaciones productores agrícolas, SAC (Sociedad de Agricultores de Colombia.

6. Gases que generan el efecto invernadero. Desde el inicio de la revolución industrial a mediados del siglo XVIII y debido a las actividades humanas, el planeta ha venido experimentando un aumento constante en las concentraciones atmosféricas de óxido nitroso (N_2O), dióxido de carbono (CO_2) y metano (CH_4), los cuales son considerados como los principales gases de efecto invernadero (Le Treut et al., 2007). Estos gases de efecto invernadero (GEI) representan menos del 1% de la atmósfera, pero este pequeño porcentaje es suficiente para producir un efecto invernadero natural que puede mantener el planeta unos 30 °C más caliente que en su ausencia y es esencial para la vida.

7. Gas metano, CH_4, o gas de los pantanos. Cierta cantidad de metano en la atmósfera, es algo normal y de hecho es bueno. El metano retiene el calor en la atmósfera y ayuda a mantener un ambiente cálido, el problema es cuando hay demasiado

metano: las capas de gases de invernadero se vuelven más grandes y espesas, reteniendo más y más calor de forma excesiva, literalmente, cocinando el planeta. Los gases (flatulencias, eructo) que cada res libera al día se estima en 300 litros de metano, lo que sumado a los generados por el rebaño mundial de rumiantes, incluidas ovejas y cabras, suponen el 18% de las emisiones internacionales de gases de efecto invernadero. En su conjunto, la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO) calcula que el sector ganadero genera más gases de efecto invernadero que el sector de transporte. (http://www.fao.org/Newsroom/es/news/2006/1000448/index.html., Christopher Mathews Oficina de prensa, FAO) "El ganado es uno de los principales responsables de los graves problemas medioambientales de hoy en día. Se requiere una acción urgente para hacer frente a esta situación", asegura Henning Steinfeld, Jefe de la Subdirección de Información Ganadera y de Análisis y Política del Sector de la FAO, y uno de los autores del estudio.

8. Combustión de biomasa. La combustión de biomasa de plantas es otra fuente importante de contaminantes del aire que incluyen dióxido de carbono, óxido nitroso y partículas de humo. Se estima que los seres humanos son responsables del 90% aproximadamente de la combustión de biomasa, principalmente, a través de la quema deliberada de vegetación forestal, asociada con la deforestación, a la quema de residuos de pastos y cultivos para favorecer el crecimiento de nuevos rebrotes y destruir hábitat de insectos dañinos. Los enormes incendios forestales que se produjeron en el Asia meridional en 1997 quemaron al menos 4,5 millones de has, y cubrieron la región con un manto de humo y neblina.

9. Los humedales y la eutrofización: Las fuentes de CH4 atmosférico son principalmente de origen biológico (70-80%) y los humedales son considerados como importantes fuentes emisores de este gas, contribuyendo con el 40-55% de las emisiones anuales globales (Bodelier and Laanbroek, 2004.) La emisión de metano a la atmosfera en los humedales no solo depende de la metanogenesis (origen del metano) sino también de la oxidación y del transporte del sedimento a la atmosfera (Christensen et al., 2003; Tauchnitz et al., 2008).

Eutrofización: Incremento de sustancias nutritivas en aguas dulces de lagos y embalses, que provoca un exceso de fitoplancton. La eutrofización de cuerpos hídricos incrementa la producción primaria autóctona, lo que genera ausencia de O2 y consecuentemente pueden predominar los procesos biológicos anaerobios, aumentando así la generación de CH4 (Liikanen and Martikainen, 2003).

Para concluir, es conveniente señalar, que la concepción y globalización del cambio climático, ha sido preocupación de las organizaciones mundiales y, de científicos e investigadores del clima y del ambiente desde 1972, por los efectos negativos que este puede generar sobre la naturaleza, la sociedad humana y la seguridad alimentaria. La primera organización fue la comisión bundtlas. Desde la fecha antes mencionada, hasta la actualidad, han trascurrido 45 años. Durante ese período, se han sucedido alrededor de 10 reuniones mundiales especializadas, con la participación de cientos de expertos, para tratar el tópico del cambio climático. La respuesta siempre es la misma, sí, se va hacer algo, pero hasta el momento, no hay respuestas medibles y positivas.

El país hoy en día, está capacitado para investigar, manejar y decidir sobre el cambio climático, en su territorio, siempre y cuando haya interés de respuesta, por parte de una sociedad que contamina el ambiente.

I.A. César Mendoza de Armas PhD.
EF. EAFIT Sorbona, Paris I, Francia

I.A. Gustavo Jiménez Narváez
Coordinador Oficina del Egresado
Facultad de Ciencias Agrarias
U.N, Sede Medellín

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Revista Facultad Nacional de Agronomía

Inheritance of lethal gene 'luteus-Pa' from cacao progenies obtained by self and cross-fertilization

UNIVERSIDAD NACIONAL DE COLOMBIA

Herencia del gen letal 'luteus-Pa' en progenies de cacao obtenidas por autofecundación y fecundación cruzada

doi: 10.15446/rfna.v70n2.64502

Zurel N. Meza S.[1], Luis F. García[1]* and Patricia V. García[1]

ABSTRACT

Keywords:
Cacao
Self-cross pollination
Lethal factor
Mendelian segregation
Chi-square (X^2) test

With the aim to study the inheritance of lethal gene 'luteus-Pa' in three derivated progenies from self and cross-fertilization of cacao (*Theobroma cacao* L) varieties, a study was carried out during january to september, 2013. As genetic material C-25, S-5 y Pa-150 varieties were utilized and this one were self and cross pollinated and their progenies were divided in two replicates with 100 seeds each and sowed in plastic recipients containing a substrate 2:1(soil: sand). In order to contrast the observed segregation with its expected homologous 3:1 (monogenic inheritance), the Chi-square (X^2) test was utilized. The results shown that only one from self-fertilized progeny of C-25 variety exhibited the mendelian segregation 3:1 in both replicates, so that it lead to infer this one is carrier of lethal gene 'luteus-Pa' in heterozigote condition whereas that the progenie from Pa-150 x C-25 cross no found none lethal seedling and submitted to Chi-square (X^2) test it could be to infer this one no segregate in the proportion 3:1 therefore, the Pa-150 variety is no carrier of this lethal gene. A better comprehension on photobiological origin, supported by molecular markers and the functional genomics of this mutant can help to identify and understand the factors involve in the photosynthetic mechanisms.

RESUMEN

Palabras claves:
Cacao
Auto-alopolinización
Factor letal
Segregación mendeliana
Prueba chi-cuadrado (X^2)

Con la finalidad de estudiar la herencia del gen letal 'luteus-Pa' en progenies derivadas de autofecundación y fecundación cruzada de tres variedades de cacao (*Theobroma cacao* L), se realizó este ensayo durante enero a septiembre, 2013. Como material genético se utilizó las variedades C-25, S-5 y Pa-150 que fueron auto y alopolinizados y sus progenies fueron divididas en 2 repeticiones de 100 semillas cada una y sembradas en contenedores plásticos conteniendo un sustrato 2:1 (suelo:arena). Para contrastar la segregación observada con la esperada 3:1 (herencia monogénica), se utilizó la prueba Chi-cuadrado (X^2). Los resultados muestran que sólo la progenie autofecundada de la variedad C-25 exhibió la segregación mendeliana 3:1 en ambas repeticiones, de modo que se puede inferir que esta variedad es portadora del gen letal 'luteus-Pa'en condición hererocigota; mientras que en la progenie de la cruza Pa-150 x C-25 al no encontrarse ninguna plántula letal y sometida a la prueba Chi-cuadrado (X^2), se infiere que falla en segregar en una proporción 3:1; por consiguiente, la variedad Pa-150 no es portadora de este gen letal. Una mejor comprensión sobre el origen fotobiológico apoyado por marcadores moleculares y la genómica funcional de este mutante, puede ayudar a identificar y comprender mejor los factores involucrados en el mecanismo fotosintético.

[1] Facultad de Agronomía. Universidad Nacional Agraria de la Selva (UNAS). Av. Universitaria - Km. 1.5 Carretera Huánuco. Tingo María, Perú
* Corresponding author <lugarc01770@gmail.com>

Received: August 13, 2016; Accepted: April 3, 2017
Rev.Fac.Nac.Agron. 70(2): 8123-8128. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

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Cacao (*Theobroma cacao* L.) is a species native to the tropical rain forests of South America whose beans are the basic input for the chocolate industry and derivatives, cosmetic and pharmaceutical industries. Wild and cultivated cacao populations exhibit genetic variability due to sexual reproduction and natural cross-pollination. The pressure of evolutionary forces, particularly, the mutations have given rise to recessive genes that have survived for generations in heterozygous condition. Mutations occur at very low frequencies modifying the genotype causing unfeasibility of the embryo or premature death of some seedlings. In cacao seedlings from Parinari series was observed leaf chlorosis, followed of necrosis and finally death of seedling, at approximately 30 to 40 days (Almeida *et al.,* 1998). It has been reported that Pa-30, Pa-121 and Pa-169 varieties when they were self or cross-fertilized and their progenies subjected to Chi-square (X^2) test, segregated in a ratio 3:1 (normal: lethal), inferring that are heterozygous genotypes carrying the lethal gene 'luteus-Pa' (Bartley *et al.,* 1983) and this gene is expressed only in recessive homozygous, being a character of monogenic inheritance (Bartley, 1969). Self-pollination or alopolinización between carriers' heterozygous trees produce a phenotypic ratio 3:1 (normal and lethal) to the emergency and a ratio 2:1 (2 normal heterozygotes and 1 dominant homozygote) to adulthood (Yamada, 1982). Another study using six varieties from Parinari series pollinated with Pa-169 variety (carrying the lethal gene 'luteus-Pa'), only Pa-44 variety showed segregation 3:1 verifying again monogenic inheritance (Yamada *et al*., 2005). A physiological study of cacao seedlings carriers of lethal gene 'luteus-Pa', identified differences between normal and mutant seedlings at 15 days after sowing. Variations in growth and subsequent death of seedlings to 60 days were related to the depletion of reserves from cotyledons, inactivity of the photosynthetic apparatus and oxidative stress (Rehem *et al*., 2011). Cacao as allogamous species, the lethal gene flow among populations can increase the frequency of this gene in the progeny and may become a limiting factor for mass propagation of seeds as rootstocks increasing production costs in the nursery and the inevitable replant new seeds. In some populations of common cacao exist heterozygous trees carriers of lethal gene 'luteus-Pa' who may have inherited from some ancient variety from Parinari series. The lethal gene 'luteus-Pa' may be useful for identifying seedlings belonging to the Pa series, in order to determine relationships between genotypes constituting a potential genetic marker and a tool for paternity tests (Bartley, 2005; Rehem *et al.,* 2010). The aim of this study was to verify the type of inheritance of the 'luteus-Pa' gene in progenies resulting from self and cross fertilization of three varieties from Peruvian cacao.

MATERIALS AND METHODS

The study involved three places: EEA-Tulumayo, genebank cacao and deckhouse mesh of the Agronomy Faculty (Tingo María). The experimental soil consisted of a mixture of organic substrate of forest and sand (ratio 2:1). The internal temperature ranged from 22.2 to 25.6 °C at 08: 00h and from 28.4 to 34.5 ºC at 14:00 pm with a brightness of 65% and 85% of relative humidity. The water requirements of the seedlings were satisfied with intraday irrigation. The genetic material consisted of three clones: S-5, C-25 and Pa-150, and this one had two components: selfing (S-5 Ø and C-25 Ø) and outcrossing (Pa-150 x C-25), utilizing eight pods from five trees for each clone. The seeds of the resulting progeny were planted in plastic containers and placed in the deckhouse mesh. The number of seeds per treatment was 200 (two replications of 100 seeds each one) sowed in four trays 25 seeds per container. For statistical analysis, the Chi-square test (X^2) was chosen in order to test hypotheses about the kind of segregation from resulting progeny to one or a few pairs of genes of Mendelian inheritance. Its formula is as follows:

$$X_c^2 = \sum_{i-1}^{c} \frac{(Oi - Ei)^2}{Ei}$$

where:
Oi = i-th observed data
Ei = i-th data expected
C = number of phenotypic classes
The X_c^2 value will be contrasted with the $X_{t\ (0.05,\ 1\ df)}^2$ value to test Ho.

The methodology used in the self-pollinations and cross-pollination involved (i) the choice of flower bud and isolation. To this purpose, 20 clear plastic tubes properly packed were placed above the flower buds and fixed to the tree until anthesis and hand pollination, (ii) for the self-pollinations, pollen from a drawn flower on the same tree was applied and then it was covered with the protective tube for eight days to avoid contamination with foreign

pollen, and among eight to ten days the fruit was covered with a transparent plastic bag cutted in the bottom ends to evacuate transpired water and protect of the infection from fungi spores and (iii) cross-pollination, it was similar to that described for the self-pollinations, with the difference in the pollen donor parent. Pollen receptors flowers corresponded to Pa-150 variety and as pollen donors to C-25 variety. Monitoring the growth of fruits fertilized from self and cross pollination was performed every two weeks during six months. At harvest the fruits were labeled, seeds were washed with sawdust to remove the mucilage and then disinfected with Cupravit (5 g kg^{-1} seed) just before sowing. The date of sowing and seedling emergence, the identifying of emergent seedlings: normal and chlorotic (lethal) and intraday monitoring of chlorosis evolution until the senescence and death of mutant plants (lethal), were recorded.

RESULTS AND DISCUSSION

Chi-square test (X^2) for progenies from selfing

The Table 1, shows that the S-5 progeny resulting from self-pollination, no exhibited none lethal seedling, being all normal. The X_c^2 = 33.333 value was higher to $X^2_{t\ (0.05,\ 1df)}$ = 3.841 value, and as falls out of the region of H_o acceptation, so we inferring this progeny not be adjust to ratio 3:1 (normal: lethal) of the expected Mendelian segregation

Table 1. Chi-square (X^2) test for cacao segregating progenies from selfing S-5 variety.

Phenotype	Segregation	Observed value (O)	Espected value (E)	O - E	$(O - E)^2 / E$
Normal	3	100	75	25	8.333
Lethal	1	0	25	-25	25.000
	TOTAL	100	100		Xc^2 = 33.333

and consequently, this genotype is not carrier of lethal gene 'luteus-Pa' in heterozygote condition.

Instead in Table 2, the progeny of the C-25 variety resulting from self-pollination, the segregation of seedlings: normal and lethal in a ratio 3:1 was observed. On average 100 emergent seedlings (replicate 1), 71 seedlings exhibited normal green colour (synthetizing chlorophyll) and 29 seedlings not exhibited (non-synthetizing chlorophyll). The X_c^2 = 0.853 value was inferior to $X^2_{t\ (0.05,\ 1df)}$ = 3.841 value and as falls out of the region of acceptation of Ho, it can be concluded that observed segregation ratio in the resulting progeny from selfing C-25 variety, it fits the pattern of expected Mendelian segregation 3:1. Likewise, similar X_c^2 = 0.853 values were obtained in the replicate 2. So, it can be inferred that C-25 variety is carrier

Table 2. Chi-square (X^2) test for cacao segregating progenies from selfing of C-25 variety.

Phenotype	Segregation	Observed value (O)		Espected value (E)	O - E		$(O - E)^2 / E$	
		R 1	R 2		R 1	R 2	R 1	R 2
Normal	3	71	79	75	-4	4	0.213	0.213
Lethal	1	29	21	25	4	-4	0.640	0.640
	TOTAL	100	100	100		Xc^2 =	0.853	0.853

R1= replicate 1; R2= replicate 2

of the lethal gene 'luteus-Pa' in heterozygote condition (LPlp) and the lethal seedlings are of genotype recessive homozygote (lp lp).

Similar results of segregation of normal and lethal seedlings ("chlorophyll deficient mutants") in a phenotypic 3:1 ratio from selfing have been reported for cacao varieties from Parinari series (Yamada,1982; Bartley *et al.,* 1983), corroborating the monogenic inheritance of lethal gene 'luteus-Pa' and identifying to the C-25 variety as a carrier genotype of this lethal gene. We believe that C-25 may be inherited the 'luteus-Pa' gene from cultivar

Pa-169 already identified as carrier and this one might have participated in different crosses in cacao breeding programs in Brazil and whose hybrid seed mixed with others hybrids it would arrived in Peru in the late 80s or earlier decades from Brazil, being found in some farms of Huánuco, San Martin and Rio Apurimac-Ene valley. Lethal seedlings ("mutants deficient in chlorophyll") die shortly after emergence because chlorophyll is essential for photosynthesis processes and also in the absorbance and transference light energy to the reaction centers within of the chloroplasts (Ning *et al*., 2013). His acute deficiency causes stunted growth, senescence and subsequent death. Although the genetic mechanism is not known, it has been reported that the yellow-pale (lethal) phenotype could be due to a problem that arises in the membranes of thylakoid which is essential for the photosynthetic process (Reed *et al*., 2014).

Chi-square test (X^2) for the progenies of cross-fertilization

The Table 3 show the results of the segregation of cacao seedlings from Pa-150 x C-25 cross. We can observe there the absence of segregation of normal and lethal seedlings in a phenotypic ratio 3:1 and being all (100%) of normal green colour and 0% abnormal (yellowish). The $\chi_c^2 = 33.333$ value was higher to $X_{t\ (0.05,\ 1df)}^2 = 3.841$ and as falls out of the Ho acceptance region, we can conclude which this obtained progeny by cross-fertilization does not adjust to expected Mendelian segregation 3:1 (normal: lethal), rejecting the Ho (3:1ratio). These results allow us to infer that Pa-150 variety (Parinari series) does not carry the lethal gene 'luteus-Pa' but the C-25 variety does; otherwise, they would have appeared as lethal seedlings in a 25% (expected frequency). Reports from Brazil admit that not all varieties from Parinari series are carriers of this lethal gene 'luteus-Pa', with exception of the Pa-169 variety (Yamada, 1982; Bartley *et al*., 1983). Generally recessive lethal alleles have variable frequencies in the populations and they can persist over a long period of time. Natural selection eliminates copies of allele when they occur in homozygote genotypes; however, recessive lethal alleles are suppressed by dominant alleles in heterozygote genotypes, and they evade natural selection; hence heterozygote carriers of recessive lethal gene have a selective advantage in the natural selection process (Ryder *et al*., 1999).

Table 3. Chi-square (X^2) test for segregating progenies of cacao of the Pa-150 x C-25 cross.

Phenotype	Segregation	Observed value (O)	Espected value (E)	O - E	$(O - E)^2 / E$
Normal	3	100	75	25	8.333
Letal	1	0	25	-25	25.000
	TOTAL	100	100		$\chi_c^2 = 33.333$

Physiogenetics of evolution of the chlorosis from lethal seedlings

Chlorophyll deficiency or albinism is perhaps the most easily mutant phenotype detectable in higher plants and in *Arabidopsis thaliana*. Among the various mutants of normal green leaves, e.g. Chlorina mutant (green-yellowing), the physiology and genetic mechanism is different from others mutants (Jarvis *et al*., 2000). The effects of mutations on chloroplast development, photosynthesis, accumulation of chlorophyll b, and levels of uptake of light by chlorophyll a/b associated proteins, have been examined by Kosuge *et al*. (2011). Conditional lethal plants have been obtained by inactivation of the ribulose-1,5-bifosfatocarboxilasa-oxygenase enzyme catalyzing both oxidation and carboxylation of ribulose-1,5-bisphosphate resulting in uptake of O_2, CO_2 release (photorespiration) and NH_4 (Migge and Becker, 2000). In the Figure 1, the evolution of lethal chlorosis in seedlings from selfing C-25 variety carrying the lethal gene 'luteus-Pa' is showed. There we can see the chlorotic leaf yellowing followed by necrosis, senescence and death of the seedlings in a period which range among12 to 28 days after emergence. According to Rehem *et al*. (2011), the leaf yellowing is a symptom of senescence associated with chlorophyll degradation and catabolized by polyphenol oxidase, among others. When occur the accumulation of anthocianins in the

senescents leaves, this is preceded by chlorophill degradation (Díaz *et al*., 2006).The persistence of the chlorophyll when the photosynthetic capacity is affected, increases susceptibility to oxidative stress induced by light in the leaf cells. A protective role of anthocyanins as sunscreens and as neutralizers for reactive oxygen species (ROS) in young expanded leaves and senescent leaves susceptible to damage by light, it has been suggested by Baker and Hardwick (1972). On the other hand, the 'shared pathway' approach to lethals gene identification, the complexity and redundancy of metabolic pathways in plants may limit the strategy overall. The assumption here is that disrupting any portion of the complex proteic will result in lethality (Meinke *et al*., 2008). Studies with molecular markers found that the level of polymorphism between Pa-30 and Pa-169, based on RAPD and SSR markers, was compatible with the average values usually found in genetic diversity studies of *T. cacao* (Rehem *et al*., 2010). Data from ESTs identified genes associated with Rubisco, peroxidases, and other proteins and enzymes related to carbon assimilation, respiration, and

Figure 1. Evolution of the chlorosis of lethal seedlings from selfing C-25 variety.

photosystem 2. Mutant seedlings were characterized by synthesizing defective PsbO and PsbA proteins, which were overexpressed from 15 to 20 days after seedling emergence (Rehem *et al*., 2016). A better understanding on the photobiological origin, the mapping with molecular markers and functional genomics of chlorophyll deficient mutants, can help to identify and understand the factors involved in the photosynthetic mechanism.

CONCLUSIONS

In the progenies from selfing, only C-25 variety showed a Mendelian segregation 3:1, identifying it as a heterozygote carrier of lethal gene 'luteus-Pa'.

The progeny from the crossing Pa-150 x C-25 does not gave none lethal seedling failing in adjust to Mendelian segregation 3:1, therefore we can to infer that the Pa-150 variety, is not carrier of lethal gene 'luteus-Pa'.

The physiogenetics basis of the chlorophyll deficient mutants are not yet very clear to understand the factors involved in the genetic control, the photosynthetic mechanism and its photobiological origin.

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Research article

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Revista Facultad Nacional de Agronomía

Genotype by environment interaction and yield stability in sugarcane

UNIVERSIDAD NACIONAL DE COLOMBIA

Interacción genotipo x ambiente y estabilidad del rendimiento en caña de azúcar

doi: 10.15446/rfna.v70n2.61790

Ramón Rea[1]*, Orlando De Sousa-Vieira[2] Alida Díaz[2], Miguel Ramón[3] and Rosaura Briceño[1]

ABSTRACT

Key words:
G x E interaction,
Phenotypic stability
Rank correlation
Saccharum spp
Hybrid

Genotype by environment interaction (GEI) reduces the association between phenotype and genotype which result in relative ranking and stability differences of genotypes across environment. The objectives of this research were (i) to select sugarcane genotypes of high yield and stable (ii) to study the interrelationships among various parametric and no parametric stability statistics. Seventeen experimental genotypes and three check cultivars of sugarcane were evaluated at seven environment using randomized completely block design. Methodologies based on analysis parametric (Regression-bi-S^2d_i, Shukla variance, Ecovalence-W, Coefficient of variation-CV, index of Lin and Binns-PI and AMMI value) and non-parametric statistics (Nassar and Huehn- $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$, $Si^{(6)}$, Kang-RS, Fox-TOP, and Thennarasu- $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$, $NPi^{(4)}$) were used for Ton of Pol per hectare (TPH). Genotypes and environment showed high significant difference (P<0.01) while GEI was significant (P<0.05). The parametric stability analysis identified the genotypes V99-236 and V00-50 as the most stable and high TPH. With non-parametric statistics were identified the genotypes V00-50, V99-236 and V98-120 as most stable. The analysis distinguished two groups of statistics using biplot: the first group (G1) formed by PI, CV, ASV, TOP, $Si^{(3)}$, $Si^{(6)}$, $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ statistics were located under the concept of dynamic stability since they are associated with TPH. The other group (G2), formed by Shukla, W, S^2d_i, bi, RS, $Si^{(2)}$, $Si^{(1)}$, $NPi^{(1)}$ statistics, fell within the static concept. Finally, genotypes V99-236 and V00-50 can be recommended as the most stable genotype in terms of both stability andTPH.

RESUMEN

Palabras claves:
Interacción G x A
Estabilidad fenotípica
Correlación de rango
Saccharum spp.
Híbrido

La interacción genotipo por ambiente (GEI) reduce la asociación entre el fenotipo y el genotipo lo cual genera cambios en el orden y en la estabilidad de genotipos a través de ambientes. Los objetivos de esta investigación fueron: (i) seleccionar genotipos de caña de azúcar de alto rendimiento y estables (ii) evaluar las interrelaciones entre diversos métodos de estabilidad paramétrica y no paramétrica. Diecisiete genotipos experimentales y tres cultivares testigos de caña de azúcar fueron evaluados en siete ambientes utilizando un diseño de bloques completamente aleatorizado. Metodologías basadas en el análisis estadístico paramétrico (Regression-bi-S^2di, varianza de Shukla, Ecovalence-W, Coeficiente de variación-CV, índice de Lin y Binns-PI y AMMI) y no paramétrico (Nassar and Huehn- $Si^{(1)}$, $Si^{(2)}$, $Si^{(3)}$, $Si^{(6)}$, Kang-RS, Fox-TOP, and Thennarasu- $NPi^{(1)}$, $NPi^{(2)}$, $NPi^{(3)}$, $NPi^{(4)}$) fueron usadas para evaluar el rendimiento en toneladas de Pol por Hectárea (TPH). Los genotipos y el ambiente mostraron diferencias estadísticamente significativa (P <0,01), mientras que la GEI fue significativo (P<0.05). Los estadísticos de estabilidad paramétricas identificaron los genotipos V99-236 y V00-50 como los más estables y de alto TPH y los no paramétricos distinguieron a los genotipos V00-50, V99-236 y V98-120. El biplot identifico dos grupos de estadísticos: El primer grupo formado por los estadísticos PI, CV, ASV, TOP, $Si^{(3)}$, $Si^{(6)}$, $NPi^{(2)}$, $NPi^{(3)}$, y $NPi^{(4)}$) que se situaron bajo el concepto de estabilidad dinámica, ya que están asociados con TPH. El otro grupo (G2), formado por los estadísticos Shukla, W, S^2di, bi, RS, $Si^{(2)}$, $Si^{(1)}$, $NPi^{(1)}$ caen dentro del concepto estabilidad estática. Finalmente, los genotipos V99-236 y V00-50 pueden ser recomendados como los más estables y de alto TPH.

[1] Fundación Instituto de Estudios Avanzados (IDEA). AP 17606, Caracas 1015-A, Venezuela.
[2] Instituto Nacional de Investigaciones Agrícolas (INIA), Estación Local Yaritagua. AP 3203, Yaracuy, Venezuela.
[3] Instituto Nacional de Investigaciones Agrícolas (INIA), Estación Local Portuguesa. AP 102, Araure, Venezuela.
* Corresponding author <ramonrea@|hotmail.com>

Received: December 28, 2016; Accepted: April 4, 2017
Rev.Fac.Nac.Agron 70(2): 8129-8138. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

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Genotype by environment interaction (GEI) is a relevant consideration for plant breeders. Sugarcane breeders usually assess a group of genotypes through environments prior to the release of a new crop for farmers' production (Rea and De Sousa-Vieira, 2002). The GEI causes the best genotype to change with the environment, and makes the selection process difficult for a particular region (Farshadfar *et al.,* 2012). Most breeders have used the term 'stability' to describe a genotype which exhibits a relatively constant yield, independent of environmental conditions. This concept of stability is in accordance with the concept of homeostasis extensively used in quantitative genetics (Becker and Leon, 1988) and may be regarded as a 'biological' or 'static' concept of stability. A genotype showing an unchanging performance in all environments does not certainly respond to improved growing conditions with increased yield. Agronomists, hence, would prefer an 'agronomic' or 'dynamic' concept of stability in which it is not indispensable that the genotypic response to environmental conditions should be identical for all genotypes (Becker and Leon, 1988).

Diverse methods have been proposed to estimate GEI or yield stability. These methods can be separated into two main groups including parametric (univariate and multivariate) and non-parametric approaches based on different strategies (Dehghani *et al.,* 2008). Univariate parametric methods have been used, such as those of Eberhart and Russell (1969), Shukla (1972) and Francis and Kannenberg (1978). These methods demand few computation and their parameters are easy to interpret biologically.

Diverse nonparametric methods have been used to describe and explain the responses of GEI (Nassar and Huehn, 1987; Kang, 1988; Fox *et al.,* 1990; Thennarasu, 1995). In this approach, no suppositions about the observations are required and there is less susceptible to measurement errors or to outliers (Huehn, 1990; Balalić *et al.*, 2011; Temesgen *et al.,* 2015; Scampin *et al.,* 2000; Rea *et al.,* 2015).

Multivariate methods such as the additive main effects and multiplicative interaction (AMMI) model has been proposed as effective methodology to predict adaptation and stability of cultivars (Guerra *et al.,* 2009). Purchase *et al.* (2000) generated the AMMI stability value (ASV) based on the AMMI model and using principal component scores (axes 1 and 2) for each genotype.

Mixed model methods also can be used to estimate GEI effects when analyzing multilocation yield trial data. If locations are random representatives of environments in the target region, the mixed model procedure stipulates best linear unbiased predictors (BLUP) of random effects. The BLUP of random effects is suitable for detecting location specific effects and estimating genotype by location interaction effects (Coutiño-Estrada and Vidal-Martínez, 2003).

The objectives of this research were (i) to select sugarcane cultivars of high cane yield (TPH) and stable through different environments in Venezuela (ii) to study the interrelationships among various parametric and no parametric phenotypic stability statistics.

MATERIAL AND METHODS

Seventeen experimental genotypes and three commercial check cultivars of sugarcane were evaluated at seven environment using randomized completely block design with three replications in the Central-Western region of Venezuela. The experimental sugarcane genotypes were: V91-1, V91-2, V91-6, V91-8, V91-15, V98-62, V98-86, V98-120, V99-117, V99-190, V99-203, V99-208, V99-213, V99-217, V99-236, V99-245 and V00-50. The check cultivars evaluated were B80-408, C323-68, and CP74-2005. All materials were evaluated at seven locations (Carora and Montaña Verde in Lara State; The Majaguas, Farm Ivonne and Farm Castillera in Portuguesa State; Santa Lucia and FUNDACANA in Yaracuy State), each with three crop-years (plant crop, first and second ratoon) during 2008-2010. Some environmental conditions of the seven experimental sites of Venezuela can be seen in Table 1. The attribute evaluated was cane yield expressed in tons of pol per hectare (TPH). The plots were three rows, with 1.5 m between rows and 10 m long. Plots were managed conventionally and followed the established local practices. All three rows were harvested for measuring cane yield (TCH). The cane was incinerated and then chopped by hand. A 10-stalk sample was randomly taken from each plot and weighed. The samples were milled and the crusher juice was analysed for sucrose content (Pol % cane). Tons of pol

per hectare (TPH=t pol ha^{-1}) was estimated as related to tons of cane per hectare (TCH) and Pol % cane by the coming formula: TPH = (TCH x Pol % cane)/100 (Guerra *et al.,* 2009).

Table 1. Principal soil and precipitation characteristics of the appraised locations

Location	Soil	Precipitation (mm)	pH
Quebrada arriba (A)	Clay loam	1101	7.7
Santa Lucia (B)	Silty clay loam	700	8.0
FUNDACAÑA (C)	Silt loam	1111	8.1
Montaña verde (D)	Loam	1048	7.3
Las Majaguas (E)	Clay loam	1500	7.0
Finca Ivone (F)	Clay loam	1500	7.0
Finca Castillera (G)	Clay loam	1500	7.0

Analysis of variance. The analysis of variance was executed contemplating the genotype and environment effects as fixed, according to the mathematics model: Yijk = μ + B/Ejk + Gi + Ej + GEij + εijk, where Yijk represents the ith genotype within the jth environment and the kth block, μ is the general mean, B/Ejk corresponds to the block within the jth environment in the kth block, Gi is the effect of the ith genotype, Ej is the effect of the jth environment, GEij is the effect of interaction of the ith genotype with the jth environment, and εijk is the effect of experimental error. The GEI was divided as stated by the additive main effect and multiplicative interaction (AMMI) models (Crossa, 1990). Data were combined over locations and analysed as combined series of RCB´s with repeated measures (crop-year) using InfoStat software (Balzarini *et al.*, 2008).

Stability analysis. Stability of the 20 genotypes for TPH was calculated by using the coefficient of regression (*b*), mean squared deviations from regression (S^2d_i), ecovalence stability index (*W*),**)** Shukla's stability variance (Shukla), Linn and Binn Index (Pi), the coefficient of variation (CV) and AMMI stability value (ASV). Several nonparametric stability statistics suggested by Nassar and Huehn (1987); Kang (1988); Fox *et al.* (1990) and Thennarasu (1995) were estimated.

The statistics based on yield ranks of genotypes (Nassar and Huehn, 1987) in each environment are expressed as follows: $S_i^{(1)}$ calculates the average of the absolute differences in the orders of a genotype in all environments, $S_i^{(2)}$ is the variance between the ranks in all environments, $S_i^{(3)}$ and $S_i^{(6)}$ are the sum of the absolute deviation and sum of squares of ranks for each genotype relative to the average of the ranks, respectively (Rea *et al.*, 2015) .

$$S_i^{(1)} = 2\sum_{j}^{n-1}\sum_{j'=j+1}^{n}\left[r_{ij} - r_{ij'}\right] / \left[n(n-1)\right]$$

$$S_i^{(2)} = 2\sum_{i=1}^{n}(r_{ij} - \overline{r}_{i.})^2 / (n-1)$$

$$S_i^{(3)} = \sum_{j=1}^{n}(r_{ij} - \overline{r}_{i.})^2 / \overline{r}_{i.}$$

$$S_i^{(6)} = \sum_{i=1}^{n}\left|r_{ij} - \overline{r}_{i.}\right|^2 / \overline{r}_{i.}$$

Where r_{ij} is the rank of the ith genotype in the jth environment, and $\overline{r}_{i.}$ is the mean rank across all environments for the ith genotype.

Thennarasu's (1995) nonparametric stability analysis considers adjusted ranks of genotypes within each test environment.

$$NP_i^{(1)} = 1/n\sum_{j=1}^{n}\left|r_{ij}^{*} - M_{di}^{*}\right|$$

$$NP_i^{(2)} = 1/n\left(\sum_{j=1}^{n}\left|r_{ij}^{*} - M_{di}^{*}\right| / M_{di}\right)$$

$$NP_i^{(3)} = \frac{\sqrt{\sum\left(r_{ij}^{*} - \overline{r}_{i.}^{*}\right)/n}}{\overline{r}_{i.}}$$

$$NP_i^{(4)} = 2/n(n-1)\left[\sum_{j=1}^{n-1}\sum_{(j'=j+1)}^{n}\left|r_{ij}^{*} - r_{ij'}^{*}\right| / \overline{r}_{i.}\right]$$

The adjusted rank r_{ij}^{*} is calculated on the basis of the adjusted phenotype values $\left(X_{ij}^{*} = X_{ij} - \overline{X}_{i.}\right)$ where $\overline{X}_{i.}$ is the mean performance of the ith genotype. The ranks, obtained from these adjusted values (X_{ij}^{*}), depend only on GEI and error effects; r_{ij}^{*} is the rank X_{ij}^{*}, $\overline{r}_{i.}^{*}$ and M_{di}^{*} are the mean and median ranks for adjusted values, while $\overline{r}_{i.}$ and M_{di} are the same parameters computed from the original (unadjusted) values.

Fox *et al.* (1990) suggested a non-parametric superiority procedure for general adaptability using stratified ranking of cultivars. A genotype that appeared mostly in the top third (high *TOP*-value) was judged a widely adapted cultivar. Kang's (1988) rank-sum (*RS*) is another non-parametric stability procedure where both yield and Shukla's (1972) stability variance were used as selection criteria. In this method, both the highest yielding genotype and the genotype with the lowest stability variance are ranked 1 and the genotype with the lowest RS value is judged the most desirable (Farshadfar *et al.,* 2012; Rea *et al.*, 2015).

Additionally, Spearman correlation coefficients among stability parameters and principal component analyses (PCA) based on the correlation matrix were executed to achieve an understanding of the association among stability parameter. All of the analyses were effectuated using InfoStat software (Balzarini *et al.*, 2008) and Agricolae program (De Mendiburu, 2015) which was originated in R software (CRAN).

RESULTS AND DISCUSSION

Analysis of GE interaction

Plant breeders inevitably face GEI once testing varieties across a number of environments. Reckoning on the magnitude of the interactions or the differential genotypic responses to environment, the varietal rankings can dissent greatly across environment (Dehghani *et al.*, 2008; Crossa, 1990). Bartlett´s homogeneity test evidenced that the mean squares of individual environments were unvarying and so the combine analysis of variance was performed. The combined analysis of variance (ANOVA) for TPH is shown in Table 2. Genotypes and environment showed high significant difference (P<0.01) while GEI was significant (P<0.05) indicating rank difference in genotypes response at different environments and the need for extension of stability analysis.

Table 2. AMMI analysis for TPH (t pol ha^{-1}) of twenty sugarcane genotypes in seven environments.

Fuentes de Variación	GL	SSAMMI	MS-AMMI	% SS
E/Rep	14	470.82	33.63	
Genotype (G)	19	5620.01	295.79**	53.07
Environment (E)	6	2700.16	450.03**	25.50
G x E (GE)	114	2270.12	19.91*	21.43
PCA 1	24	234.025	9.75	31.00
PCA 2	22	146.864	6.68	19.42

*, ** significant at 5% and 1% probability level by F test, respectively

The mean TPH across environments over three years (Table 3) showed considerable changes in ranks among the genotypes, reflective the presence of high GE interactions (Rea *et al.*, 2015). These results show that the heterogeneousness of the environments and genetic variability of the genotypes becomes manifest. The environment mean yield ranged from 13.12 TPH in Santa Lucia to 17.56 TPH in Montaña Verde indicating differences among test environments. The highest yield 23.27 TPH was obtained from genotype V98-120 at

Montaña Verde, while the lowest was 8.26 TPH from genotype V91-6 at Santa Lucia. The results of the non-parametric and parametric statistics and their ranks are presented in Table 4 and 5, respectively.

Table 3. Mean yield (TPH) of twenty sugarcane genotypes tested across crops in seven environments.

Genotype/ Location	Quebrada Arriba	Santa Lucia	FUNDACANA	Montaña Verde	The Majaguas	Ivonne	Castillera
B80-408	13.90	11.93	16.30	17.92	16.44	15.00	14.17
C323-68	14.99	13.40	17.11	20.27	18.19	15.66	13.98
CP74-2005	14.97	12.11	14.00	15.1	15.54	12.84	12.34
V00-50	18.29	14.46	17.08	20.62	19.28	15.69	16.6
V91-1	15.19	13.10	14.61	18.68	12.31	13.79	10.84
V91-15	10.09	11.62	11.85	16.01	13.85	11.06	12.29
V91-2	12.03	10.43	13.38	13.12	12.71	9.88	8.63
V91-6	15.36	8.26	14.18	15.17	13.86	12.17	11.56
V91-8	14,24	12.19	11.09	14.03	12.84	12.04	11.62
V98-120	18.31	15.46	16.57	23.27	17.16	14.01	16.06
V98-62	19.64	14.87	17.43	21.38	19.01	13.73	13.44
V98-86	14.39	12.68	11.64	14.36	14.64	14.71	12.02
V99-117	13.04	11.53	15.96	14.16	14.09	11.25	12.44
V99-190	18.48	16.81	14.67	18.06	14.22	13.73	15.27
V99-203	17.52	16.20	14.23	16.06	15.07	14.27	12.49
V99-208	19.78	15.29	14.93	19.93	18.80	14.65	16.76
V99-213	15.70	14.60	18.03	21.49	18.12	15.08	16.47
V99-217	12.80	10.93	10.20	14.27	10.99	13.51	11.53
V99-236	20.78	16.68	20.44	20.08	18.78	15.72	16.24
V99-245	12.46	9.78	14.46	17.17	9.55	11.20	12.89
Mean Location	15.60	13.12	14.91	17.56	15.27	13.50	13.38

Univariate stability. In Table 4 is presented the mean yield values (TPH) and stability parameters. Finlay and Wilkinson (1963) and Eberhart and Russell (1966) considered genotypes with high mean yield, coefficients of regression equivalent to unity (bi= 1) and deviation from regression proximate zero (S^2d_i=0) to be stable. According to these parameters only V99-236 and V00-50 genotypes meet these conditions. Francis and Kannenberg (1978) defined a stable genotype as one that provides high yield and constant performance across locations. In conformity with this definition, V99-236, V99-190, V99-213, V98-62 and V98-120 are considered stable since that presented low CV and high TPH. Rea and De Sousa-Vieira (2002) used this method and concluded that the CV could be used to identify genotypes on a group basis rather than individually; however, the method can also be used in a plant-breeding context. In the method of Lin and Binns (1988) the best genotype is considered to be the one with the greatest performance and the lowest value of Pi. Here, we found that genotypes V99-236, V00-50, V98-120, V98-62, and V99-208 showed lower Pi values, indicating greater adaptability to these environments. Wricke (1962) recommended using ecovalance (W) as a stability parameter. Genotypes with the smallest ecovalance (W) values are contemplated stable. The W was lowest for genotypes CP74-2005, V00-50 and V99-236. Shukla (1972) defined a stability variance value, which considered a genotype with relatively large variance to have low stability. By Shukla´s definition,

genotypes CP74-2005, V00-50 and V99-236 ranked the most highly stables. Considering all methods of univariate stability can conclude that genotypes V99-236 and V00-50 were the most stable and high yields.

Multivariate stability. The AMMI model does not make specification for a quantitative stability measure, and as such a measure is important in order to quantify and sort genotypes in terms of yield stability. Hence, AMMI stability value **(**ASV) suggested by Purchase *et al* (2000) was applied to quantify and rank genotypes according to their yield stability. The genotype with the lowest ASV value is taken as most stable. Consequently, genotypes V99-236, CP74-2005 and B80-408 were the most stable (Table 4).

Table 4. Mean yield values (TPH) and stability parameters of twenty sugarcane genotypes across seven environments.

Genotypes	TPH	PCA1	PCA2	ASV	*bi*	S^2d_i	CV	W	Shukla	Pi
V91-1	14.07	0.02	0.94	6.95	1.29	1.57	32.60	40.58	7.15	187.54
V91-2	11.45	-0.09	0.39	6.26	0.91	1.23	27.33	22.91	3.87	413.82
V91-6	12.94	-0.36	0.49	3.64	1.30	1.57	30.57	40.93	7.21	276.37
V91-8	12.58	0.88	-0.25	5.12	0.53	0.92	28.18	29.56	5.11	318.45
V91-15	12.39	-0.44	-0.93	4.98	0.80	1.65	37.73	42.89	7.57	339.22
V98-62	17.07	-0.25	1.37	4.84	1.87	1.06	28.71	51.23	9.12	32.76
V98-86	13.49	0.90	-0.71	3.94	0.36	1.32	25.69	44.92	7.95	259.88
V98-120	17.26	-0.45	0.41	5.18	1.75	1.19	28.81	46.37	8.23	30.85
V99-117	13.21	-0.39	0.06	3.49	0.64	1.44	30.47	36.59	6.41	256.55
V99-190	15.89	1.09	0.13	4.67	0.62	1.78	26.85	54.16	9.66	97.54
V99-203	15.12	1.26	0.21	4.65	0.49	1.59	32.07	49.31	8.76	139.80
V99-208	17.16	0.49	0.45	5.32	1.15	1.57	29.50	38.01	6.67	46.26
V99-213	17.07	-0.97	-0.31	5.51	1.29	1.35	27.52	30.83	5.34	41.55
V99-217	12.03	0.73	-1.07	5.82	0.45	1.46	29.71	45.64	8.08	385.28
V99-236	18.39	0.03	-0.01	1.34	1.09	1.40	26.30	22.51	3.80	10.73
V99-245	12.50	-0.77	-0.49	5.41	1.16	2.17	38.61	71.51	12.87	321.91
V00-50	17.43	-0.31	0.14	5.23	1.25	0.84	31.72	13.44	2.12	30.06
B80-408	15.10	-0.74	-0.60	2.99	0.96	1.40	28.58	29.47	5.09	132.13
C323-68	16.23	-0.86	-0.29	4.15	1.32	1.38	28.54	33.41	5.82	74.26
CP74-2005	13.84	0.22	0.10	1.90	0.77	0.79	30.21	11.85	1.83	206.08

Genotypes	Pi	TOP	RS	$Si^{(1)}$	$Si^{(2)}$	$Si^{(3)}$	$Si^{(6)}$	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$	$NPi^{(4)}$
V91-1	187.54	0	22	0.71	44.14	13.02	2.85	4.86	0.44	0.71	0.08
V91-2	413.82	0	24	0.29	58.62	6.47	3.88	4.00	2.00	1.90	0.12
V91-6	276.37	0	27	0.24	29.81	10.73	2.50	4.00	0.57	0.80	0.04
V91-8	318.45	0	19	0.24	30.57	7.60	2.40	4.57	0.91	1.02	0.05
V91-15	339.22	0	31	0.43	38.00	8.41	3.12	4.57	0.76	1.17	0.09
V98-62	32.76	4	23	0.00	24.81	5.07	1.33	6.29	0.35	0.45	0.00
V98-86	259.88	0	27	0.52	44.90	11.65	2.60	5.14	0.64	0.76	0.06
V98-120	30.85	1	19	0.38	32.48	2.38	0.78	3.86	0.24	0.34	0.02
V99-117	256.55	0	23	0.33	34.00	10.94	2.94	4.43	0.89	0.80	0.05
V99-190	97.54	1	27	0.48	47.95	7.59	1.79	5.71	0.48	0.48	0.04
V99-203	139.80	1	26	0.43	45.95	5.98	1.59	5.29	0.48	0.53	0.04
V99-208	46.26	3	14	0.67	52.14	3.27	1.06	5.86	0.37	0.41	0.04
V99-213	41.55	3	13	0.48	32.90	2.17	0.89	4.71	0.28	0.32	0.03
V99-217	385.28	0	34	0.14	40.57	7.92	2.77	5.00	1.25	1.59	0.04
V99-236	10.73	4	7	0.14	30.81	1.28	0.64	4.14	0.22	0.28	0.01
V99-245	321.91	0	37	0.43	58.95	19.33	4.77	6.43	2.14	1.28	0.08
V00-50	30.06	3	4	0.24	22.62	2.24	0.82	3.43	0.20	0.26	0.01
B80-408	132.13	0	15	0.29	34.33	7.41	1.85	4.14	0.32	0.47	0.02
C323-68	74.26	1	15	0.48	44.24	3.55	1.20	5.43	0.34	0.42	0.03
CP74-2005	206.08	0	13	0.24	17.62	3.25	1.25	3.00	0.38	0.49	0.03

Non parametric stability. The results of 10 nonparametric stability measures and genotypes mean yield are resumed in Table 4. The $Si^{(1)}$ and $Si^{(2)}$ statistics are based on ranks of genotypes across locations and they give proportional weight to each environment. Genotypes with less modification in rank are considered to be more stable. According to both $Si^{(1)}$ and $Si^{(2)}$ V99-236 and V00-50 had the smallest changes in ranks and are thus considered as the most stable genotypes. Two other non-parametric statistics, $Si^{(3)}$ and $Si^{(6)}$, integrate yield and stability based on yield orders of genotypes in each environment (Nassar and Huehn, 1987). The lowest value for each of these statistics reveals maximal stability for a certain genotype. Genotypes V99-236, V00-50, V98-120, V99-208, V99-213 had the lowest $Si^{(3)}$ and $Si^{(6)}$ values hence, these genotypes were identified as the most stable genotypes.

Results for Thennarasu´s (1995) non-parametric stability statistics, estimate from ranks of adjusted yield means, are showed in Table 4. The ranks of genotypes based on these statistics are presented in Table 5. According to $NPi^{(1)}$, genotypes CP74-2005, V00-50, V98-120 were stable in analogy with the other genotypes. Genotypes V00-50, V99-236, V98-120, V99-236 had the lowest value of $NPi^{(2)}$ and were judged stable. $NPi^{(3)}$ and $NPi^{(4)}$ also recognized genotypes V00-50, V99-236 and V98-120 how the most stable genotypes and high mean yield. Kang´s (1988) non-parametric stability statistic (RS) applies both yield and stability variance (Shukla, 1972) with the genotype having the lowest rank-sum being the most promising. In this case, the genotypes V00-50, V99-236, V99-213, and CP74-2005 had the lowest values, and were stable genotypes with high yield in comparison with other genotypes. Non-parametric (TOP) superiority measure (Fox *et al*., 1990) identified genotypes V99-236, V98-62, V00-50 and V99-208 presented mainly in the top third, thus, these genotypes were stable. Similarly, Segherloo *et al*. (2008) found a highly significant association between mean yield and Fox-rank.

Table 5. Ranks of twenty genotypes across environments using stability statistics

Genotypes	TPH	ASV	*bi*	S^2d_i	CV	W	Shukla	Pi	TOP	RS	$Si^{(1)}$	$Si^{(2)}$	$Si^{(3)}$	$Si^{(6)}$	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$	$NPi^{(4)}$
V91-1	11	20	10	15	18	11	11	11	8	10	20	13	19	16	12	10	12	17
V91-2	20	19	3	6	4	4	4	20	8	13	8	19	10	19	4	19	20	20
V91-6	15	5	11	16	15	12	12	15	8	15	4	4	16	13	4	13	14	9
V91-8	16	12	15	3	6	6	6	16	8	8	4	5	13	12	9	17	16	14
V91-15	18	11	6	18	19	13	13	18	8	18	12	11	15	18	9	15	17	19
V98-62	5	4	20	4	9	18	18	4	1	11	1	3	8	8	19	7	7	1
V98-86	13	6	18	7	1	14	14	14	8	15	18	15	18	14	14	14	13	16
V98-120	3	13	19	5	10	16	16	3	5	8	11	7	4	2	3	3	4	4
V99-117	14	10	13	12	14	9	9	13	8	11	10	9	17	17	8	16	14	14
V99-190	8	9	14	19	3	19	19	8	5	15	15	17	12	10	17	11	9	9
V99-203	9	8	16	17	17	17	17	10	8	14	12	16	9	9	15	11	11	9
V99-208	4	15	4	14	11	10	10	6	3	5	19	18	6	5	18	8	5	9
V99-213	6	17	9	8	5	7	7	5	8	3	15	8	2	4	11	4	3	6
V99-217	19	18	17	13	12	15	15	19	8	19	2	12	14	15	13	18	19	9
V99-236	1	1	1	10	2	3	3	1	1	2	2	6	1	1	6	2	2	2
V99-245	17	16	5	20	20	20	20	17	8	20	12	20	20	20	20	20	18	17
V00-50	2	14	8	2	16	2	2	2	3	1	4	2	3	3	2	1	1	2
B80-408	10	3	2	11	8	5	5	9	8	6	8	10	11	11	6	5	8	4
C323-68	7	7	12	9	7	8	8	7	5	6	15	14	7	6	16	6	6	6
CP74-2005	12	2	7	1	13	1	1	12	8	3	4	1	5	7	1	9	10	6

Correlation between mean yield (TPH) and stability statistics

The Spearman's rank correlations between each combination of stability measures were estimated (Table 6) and demonstrated a high positive significant rank correlation between TPH and PI, TOP, $NPi^{(2)}$, $NPi^{(3)}$, and $NPi^{(4)}$. The parameters $NPi^{(2)}$, $NPi^{(3)}$ and $NPi^{(4)}$ were associated positive with $Si^{(6)}$ and $Si^{(3)}$ with $Si^{(6)}$, RS, and $NPi^{(2)}$. Therefore, only one of these parameters would be adequate to select stable genotypes in a breeding

program (Mohammadi *et al*., 2007). Complete correlation was found between Wricke's and Shukla's statistics. Kang *et al*. (1987) indicated that Wricke´s ecovalance (W) and stability variance (Shukla) were equal; because Shukla´s stability variance is a linear combination of the ecovalence so for ranking purposes these methods are equivalent (Bujak *et al*., 2014). To better comprehend the interrelationships among the parametric and non-parametric statistics, principal component analysis, based on the correlation matrix of rank (Table 5) was used. The first and second principal components of the rank correlation accounted for 48.10% and 18.80% of the variation, respectively, making a total of 66.90% of the original variance among the stability parameters (Figure 1). Similar results have been reported from other studies in faba bean and field pea (Flores *et al*., 1998), durum

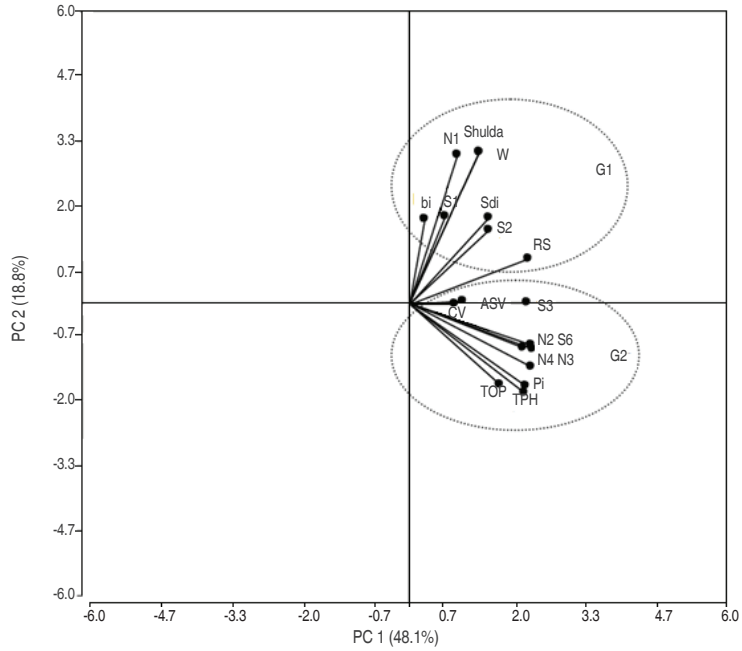

Figure 1. Biplot of IPC1 and IPC2 of the rank correlation matrix of the 17 stability parameters with mean yield (TPH).

Table 6. Spearman correlation of stability parameters and TPH in twenty sugarcane genotypes at seven environments.

Parameters	TPH	ASV	bi	Sdi	CV	W	Shukla	Pi	TOP	RS	$Si^{(1)}$	$Si^{(2)}$	$Si^{(3)}$	$Si^{(6)}$	$NPi^{(1)}$	$NPi^{(2)}$	$NPi^{(3)}$
ASV	0.28																
bi	-0.02	-0.02															
Sdi	0.27	0.16	-0.15														
CV	0.24	0.26	-0.07	0.39													
W	0.13	0.14	0.57	0.58	0.23												
Shukla	0.13	0.14	0.57	0.58	0.23	1**											
Pi	0.99**	0.3	-0.02	0.3	0.24	0.15	0.15										
TOP	0.8**	0.24	-0.02	0.23	0.27	0.004	0.004	0.79**									
RS	0.71	0.2	0.34	0.62	0.26	0.76	0.76	0.72	0.45								
$Si^{(1)}$	-0.06	0.35	-0.03	0.42	0.03	0.26	0.26	-0.004	0.16	0.1							
$Si^{(2)}$	0.3	0.43	-0.14	0.62	-0.03	0.43	0.43	0.36	0.19	0.5	0.65						
$Si^{(3)}$	0.74	0.21	0.17	0.53	0.35	0.47	0.47	0.74	0.61	0.79**	0.25	0.4					
$Si^{(6)}$	0.9	0.33	-0.03	0.45	0.33	0.3	0.3	0.89**	0.7	0.78	0.12	0.46	0.89**				
$NPi^{(1)}$	0.002	0.14	0.26	0.5	-0.003	0.68	0.68	0.03	-0.14	0.42	0.42	0.6	0.32	0.19			
$NPi^{(2)}$	0.92**	0.33	0.11	0.38	0.21	0.35	0.35	0.93**	0.64	0.8**	0.03	0.47	0.78	0.89**	0.26		
$NPi^{(3)}$	0.97**	0.3	0.07	0.34	0.26	0.27	0.27	0.98**	0.72	0.79**	-0.05	0.38	0.79**	0.93**	0.11	0.96**	
$NPi^{(4)}$	0.79**	0.5	-0.09	0.46	0.24	0.22	0.22	0.83**	0.66	0.64	0.44	0.61	0.75	0.85**	0.19	0.84**	0.82**

wheat (Kilic *et al*., 2010), barley (Mut *et al*., 2010) and sugarcane (Rea *et al*., 2015). Results of the biplot of the first two principal components based on the rank correlation matrix were more or less consistent with the Spearman rank correlation coefficients (Figure 1, Table 6). The stability parameters were separated into two stability concepts: Group 1, parameters that corresponded with the dynamic/agronomic stability concept which were associated to mean yield (TPH) such as PI, TOP, $NPi^{(3)}$, $Si^{(6)}$, $NPi^{(2)}$, $NPi^{(4)}$, $Si^{(3)}$, CV and ASV. Group 2, the remaining stability parameters corresponding with the static/ biological stability concept were assigned to RS, $Si^{(2)}$, $Si^{(1)}$, (S^2di), b*i*, Shukla, W, $NPi^{(1)}$ (Figure 1). These parameters, which identify genotypes with high phenotypic stability without due consideration of mean yield, may not be appropriate, as both breeders and farmers incline toward to select genotypes of good yield with perform regularity across environments (Mohammadi and Amri, 2008; Farshadfar *et al*., 2012; Khalili and Pour-Aboughadareh, 2016).

CONCLUSIONS

Various stability statistics were used in this research for quantifying genotype stability in relation to yield. Both yield and stability should be examined simultaneously to deal the effect of GEI and to accomplish genotype selection more accurate and refined. Several methods have been considered to analyze phenotypic stability although some of them have their limitations and there is no superior method to be recommended in all circumstances. Besides, some methodologies are optional while others are complementary, being able to be used combined. It is also recommendable to use the parametric and non-parametric stability measures jointly since results obtained from the two groups of stability measures can complement each other. Also, to capitalize on the GEI and to select breeding materials adapted to favourable and unfavourable growing conditions, selection of specific cultivars adapted to specific environments appears to be necessary. Finally, both parametric and non-parametric estimates of stability indicated either V99-936 or V00-50 as the genotype most stable and high yield.

ACKNOWLEDGEMENTS

The research was supported by the Instituto Nacional de InvestigacionesAgrícolas (INIA, Yaracuy).

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Revista Facultad Nacional de Agronomía

Diaspididae on *Citrus* spp. (Rutaceae) from Colombia: New records and a taxonomic key to their identification

Diaspididae en *Citrus* spp. (Rutaceae) de Colombia: Nuevos registros y una clave taxonómica para su identificación

doi: 10.15446/rfna.v70n2.64519

Andrea Amalia Ramos-Portilla[1,2] and Alejandro Caballero[2]*

ABSTRACT

In this manuscript *Aonidiella comperei* is reported for the first time in Colombia; the specimens were found associated with branches, leaves and fruits of *Citrus* x *latifolia* (Rutaceae) in the department of Tolima. Also we obtained physical evidence of the association of *Parlatoria ziziphi* and *Citrus* x *limonia* (Rutaceae) in Colombia from a sample collected in the field; until this paper the only record of *P. ziziphi* in the country came from specimens intercepted in a quarantine inspection at a port of entry in the United States. Field and slide-mounted characteristics are provided for *A. comperei*. Also a taxonomic key to species of Diaspididae present on *Citrus* spp. in Colombia is given.

Key words:
Coccomorpha
Invasive species
Neotropical region
New host
Pest

RESUMEN

En este manuscrito se registra por primera vez para Colombia a *Aonidiella comperei*; los especímenes se encontraron asociados a ramas, hojas y frutos de *Citrus* x *latifolia* (Rutaceae) en el departamento del Tolima. Además, se verifica la asociación *Parlatoria ziziphi* y *Citrus* x *limonia* (Rutaceae), a partir de recolecciones en campo; hasta la fecha, su único registro para el país provenía de especímenes interceptados en puertos de entrada en Estados Unidos. Se provee información de características en campo y en montaje en lámina para *A. comperei*, al igual que una clave taxonómica de especies de Diaspididae presentes en *Citrus* spp. de Colombia.

Palabras claves:
Coccomorpha
Especies invasivas
Región neotropical
Nuevos hospedantes
Plaga

[1] Dirección técnica de Sanidad Vegetal. Instituto Colombiano Agropecuario (ICA). Carrera 41 No. 17-81. Bogotá, Colombia.
[2] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. A.A. 14490, Bogotá, Colombia.
* Corresponding autor: <lacaballeror@unal.edu.co>

Received: July 22, 2016; Accepted: March 8, 2017
Rev.Fac.Nac.Agron. 70(2): 8139-8154. 2017
ISSN 0304-2847 / e-ISSN 2248-7026

The Diaspididae (Hemiptera: Coccomorpha), commonly known as "armored scale insects", is one of the most speciose families in that infraorder, and it is composed of 2650 species in 400 genera (García *et al*., 2016). Armored scales occur on a wide variety of host plants encompassing more than 1,380 plant genera in 182 plant families (Miller and Davidson, 2005). This family is particularly important in agriculture and wild vegetation (Zamudio and Claps, 2005) because it includes numerous highly prolific species that can reach high populations and become serious pests of several crops (Claps and Teran, 2001).

On *Citrus* spp. (Rutaceae) there are records of 112 species of Diaspididae (García *et al*., 2016), of which 19 have been recorded in Colombia (Balachowsky, 1959, 1959a; Figueroa, 1946, 1952, 1977; Gallego and Vélez, 1992; Kondo, 2001; Kondo *et al*., 2012; Mosquera, 1979; Posada, 1989).

The genus *Aonidiella* Berlese and Leonardi 1896 has 32 species described around the world, and includes pest species such as *Aonidiella aurantii* (Maskell), it is considered as the most injurious pest of citrus crops around the world (Miller and Davison 1990), *Aonidiella citrina* (Coquillett) in citrus (EFSA PLH Panel, 2014); *Aonidiella eremocitri* McKenzie on oil palm and coconut (Mariau, 1998); *Aonidiella gracilis* (Balachowsky) on cacao (Liegeois, 1944); *Aonidiella inornata* (McKenzie) on papaya (Lee and Wen, 1977) and mango (Chua and Wood, 1990); *Aonidiella orientalis* (Newstead) recorded as a pest of several agricultural crops such as citrus (Rose, 1990), tea (Nagarkatti and Sankaran, 1990), date palm (Rajagopal and Krishnamoorty, 1996), palms and ornamentals (Dekle, 1976), papaya (Elder *et al*., 1998) and mango (Swirski *et al*., 2002); and *Aonidiella taxus* Leonardi recorded causing damage to *Taxus* sp. and *Podocarpus* sp. trees (Miller and Davidson, 1990). In Colombia, only *A. orientalis* has been hitherto recorded (Kondo, 2001; Posada, 1989).

Before this study, *Aonidella comperei* McKenzie was recorded in 22 countries in the Neotropical and Oriental regions (García *et al*., 2016). In the Neotropics, *A. comperei* has been recorded in Brazil (Culik *et al*., 2008; Martins *et al*., 2004), Dominica (McKenzie, 1946), Guadeloupe (Balachowsky, 1957), Guatemala (McKenzie, 1946), Haiti (McKenzie, 1946) Martinique (Balachowsky, 1957), Puerto Rico (Martorell, 1976), Saint Martin (Matile and Etienne, 2006) and the U.S. Virgin Islands (Nakahara, 1983).

Aonidiella comperei is considered an important pest species of papaya, *Carica papaya* L. (Caricaceae) in Brazil because it has a widespread distribution and frequent occurrence; it causes cosmetic damage to fruit and weakens it host plant, and is also of concern as a pest of quarantine significance (Martins *et al*., 2014). It is a polyphagous species, affecting 14 plant species belonging to 12 families: *Annona muricata* L. (Annonaceae) (Martorell, 1976), *Cocos nucifera* L. (Arecaceae) (McKenzie, 1946), *Pluchea odorata* (L.) (Asteraceae) (Williams and Watson, 1988), *Carica papaya* (Martins *et al*., 2004), *Cucurbita maxima* Duchesne (Cucurbitaceae) (Velasquez, 1971), *Diospyros* sp. (Ebenaceae) (Williams and Watson, 1988), *Annesijoa* sp. (Euphorbiaceae) (Williams and Watson 1988), *Ficus* sp. (Moraceae) (Williams and Watson, 1988), *Musa* sp. (Musaceae) (Williams and Watson, 1988), *Morinda citrifolia* L. (Rubiaceae) (Williams and Watson, 1988), *Citrus aurantifolia* Swingle (McKenzie, 1946), *Citrus maxima* (Burm.) (McKenzie, 1946), *Citrus* sp. (Rutaceae) (McKenzie, 1937) and *Vitis* sp. (Vitaceae) (McKenzie, 1946).

Parlatoria ziziphi (Lucas) is a cosmopolitan species (Garcia *et al*., 2016), known to occur in Africa, Asia, Central and South America, Europe, Oceania, and the West Indies (Miller and Davidson, 2005). The species is considered to have a very limited host range, probably *Citrus* spp., *Murraya* spp., *Poncirus* spp., and *Severinia* spp., with *Citrus* spp. being the predominant host (Miller and Davidson, 2005). In Colombia this species was recorded by Blackburn and Miller (1984) based on information from the United States Department of Agriculture, without information about its hosts.

Parlatoria ziziphi is known as "the black parlatoria scale" and it has long been considered one of the major pests of citrus in certain areas (Miller and Davidson, 2005). Heavy infestations of this scale cause chlorosis and premature leaf drop, dieback of twigs and branches, stunting and distortion of fruit, and premature fruit drop and perhaps the most characteristic damage is the virtually unremovable scale cover on the fruit (Miller and Davidson, 2005). Beardsley and González (1975) and

Miller and Davidson (1990) considered this species as a serious world pest.

The purpose of this paper is to provide new biological information of *Aonidiella comperei* and *Parlatoria ziziphi*. We report for the first time *A. comperei* and *P. ziziphi* on *Citrus* spp. in Colombia. Brief diagnoses of the adult females are given for both species. An updated list and a taxonomic key of all species of Diaspididae recorded on *Citrus* spp. (Rutaceae) from Colombia is provided.

MATERIALS AND METHODS

Samples of scales insects were collected on fruits and leaves of Key lime, *Citrus* x *latifolia* Tanaka ex Q. Jiménez (Rutaceae) from commercial crops in the department of Tolima, by the first author as part of plant health surveillance activities conducted by the Colombian Agricultural Institute (ICA) and on leaves of *Citrus* x *limonia* (L.) Osbeck (Rutaceae) in the department of Atlántico by entomologist Oscar Dix.

Slide-mounted specimens were prepared according to the protocol by Siresena *et al*. (2013); however, the exposure time to which the specimens were subjected to the chemical reagents was modified. The specimens are deposited at the "Universidad Nacional Agronomía Bogotá" UNAB entomological museum, Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Sede Bogotá, Bogotá, Cundinamarca, Colombia. Imaging analysis was done under a phase contrast microscope Nikon Eclipse E600 and the software Image Pro Insight v 8.0.

Specimens were identified by checking the morphological descriptions of McKenzie (1937), Beardsley (1966), Miller and Davidson (2005) and by using the keys by Beardsley (1966), Williams and Watson (1988) and Miller and Davidson (2005). The taxonomic key of species of Diaspididae of *Citrus* spp. for Colombia is based on morphological external features of the adult female. The species included in the key correspond to those recorded on *Citrus* spp. from Colombia by Balachowsky (1959), Figueroa (1946, 1952, 1977), Gallego and Vélez (1992), Kondo (2001) and Posada (1989).

RESULTS AND DISCUSSION

Aonidiella comperei McKenzie 1937, 327. Type data: INDIA: Bombay, Calaba, on *Citrus* sp.
Chrysomphalus comperei Lindinger 1957: 545

Figure 1. Fruit (left) and branch (right) of *Citrus* x *latifolia* infested with *Aonidiella comperei*.

Field characteristics

The female scale is circular, smooth, flat, light brown in the center, surrounded by a white halo (Figure 2A). The insect is reniform, membranous and yellow when it is young (Figure 2B) and it turns orange and sclerotized at maturity (Figure 2C). The female has two nymphal instars, all of them slightly darker than the adult female (Figure 2D).

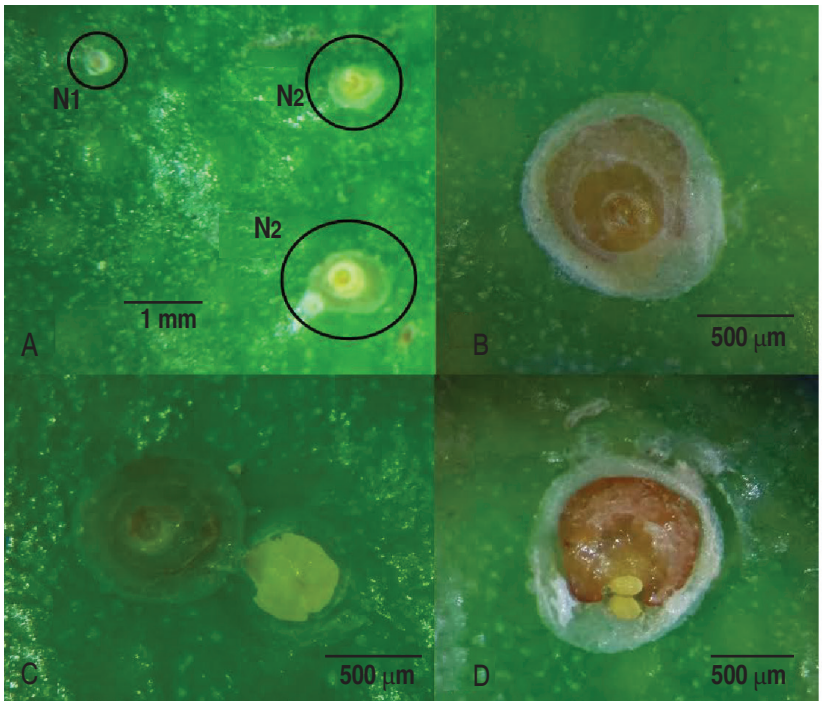

Figure 2. Live specimens of *Aonidiella comperei*. A. Two stages of development, N1: first-instar nymph, N2: second-instar nymph B. Adult female cover. C. Young adult female separated from its scale; D. Mature adult female after removal of scale.

Slide-mounted characters

The adult female is reniform, highly sclerotized at maturity (Figure 3A). The following features are given by McKenzie (1937): pygidium not heavily sclerotized; three pairs of lobes present, the median pair only slightly larger than the second pair (Figure 3B); paraphyses small, short, and slender; tubular ducts broad and conspicuous (Figure 3C); anal opening large; venter with a few small ducts, situated close to the margin of the pygidium; perivulvar pores present, in two groups only, of apparently no more than two pores in each group (Figure 3D). The differences between *A. comperei* and *A. orientalis* are listed in Table 1.

Table 1. Morphological differences between *Aonidiella orientalis* and *A. comperei*, showed as attributes. Character states taken from Beardsley (1966), Mckenzie (1937) and Williams and Watson (1988).

Character states	Attribute for *Aonidiella orientalis*	Attribute for *Aonidiella comperei*
Grade of development of prosomatic lobes	Weakly developed	Well developed (Figure 3E)
Number of rows or clusters of dorsal ducts on either side of abdominal submargin	One to three	None
Presence/absence of conspicuous groups of ducts on lateral margin of dorsum of each three prepygidial abdominal segments	Present	Absent
Form of the plates lateral to third lobe	Not fringed, each with one long fleshy process	Fringed (Figure 3F)
Number of distinguishable perivulvar pore cluster	Five	Two

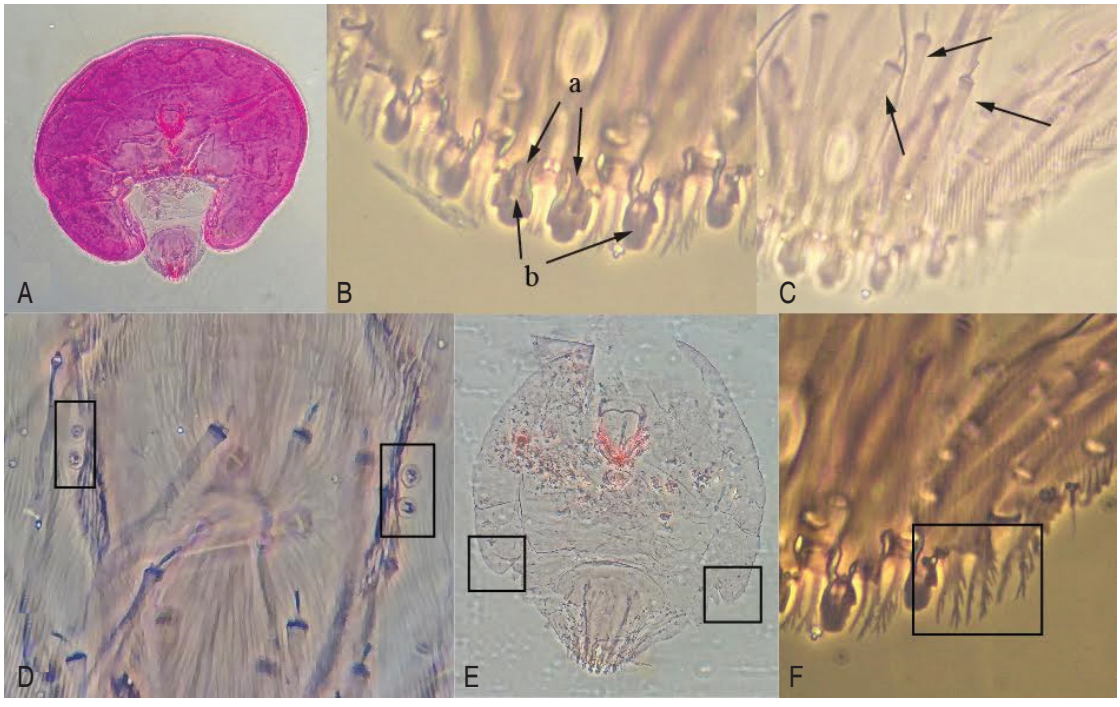

Figure 3. Slide-mounted features of *Aonidiella comperei*: A. Reniform and highly sclerotized mature adult female. B. Three pairs of lobes present on the pygidium, the median pair (a) only slightly larger than the second pair (b). C. Tubular ducts. D. Perivulvar pores in two groups, each with two pores. E. Prosomatic lobes well developed. F. Fringed plates lateral to third lobe.

Material studied

Aonidiella comperei McKenzie. Colombia, Tolima, Chicoral, vereda Las Mercedes, finca El Diamante, 318 m.a.s.l., 04°13′41.34″ N, 75°00′11.1636″ W, 18th november 2015, coll. A. Ramos ex. leaves, branches and fruits of key lime, *Citrus* x *latifolia* (Rutaceae), 32♀♀, Catalogue No. UNAB 1837; Tolima, Espinal, vereda La Morena, Citriexpinal, 331 m.a.s.l., 04°13'59.62" N, 74°54'23.96" W, 2nd march 2016, coll. A. Ramos, ex. stored fruits of key lime, *Citrus* x *latifolia* (Rutaceae). 10♀♀, Catalogue No. UNAB 1837.

NOTE: The scale insects were found in high populations (Figure 1).

Parlatoria ziziphi (Lucas)

Coccus ziziphi Lucas, 1853: xxix. Type data: FRANCE: on *Ziziphus pinnachristi*. Syntypes, female. Described: female.
Chermes aurantii Boisduval, 1867: 338-339. [Synonymized by McKenzie, 1945: 54].
Parlatoria lucassi Targioni Tozzetti, 1868: 735. Nomen nudum.
Parlatoria (*Websteriella*) *ziziphus* (Lucas); Ramakrishna Ayyar, 1919: 26. Change of combination.
Apteronidia ziziphi (Lucas); Lindinger, 1934: 62. Change of combination.
Diaspis ziziphus (Lucas); Lindinger, 1934: 62. Change of combination.
Parlatoreopsis ziziphi (Lucas); Kawai, 1972: 23. Change of combination.

Field characters

According to Miller and Davidson (2005) the adult female cover is flat, broadly elongate oval, black with narrow white fringe, with two or three longitudinal ridges, shed skins marginal, black, primary component of cover second shed skin; white; body of newly matured adult female yellow brown, margin of body lateral of mouthparts with a small lobe; normally on leaves, also on branches and fruit.

Slide-mounted characters

The description and character states that differentiates *P. ziziphi* from others species of *Parlatoria* agrees well with those proposed by Miller and Davidson (2005): body

oval, length usually less than two times the greatest width; presence of perivulvar pores; marginal macroducts barrel shaped, length of ducts usually less than three times width of inner end of duct; a barrel shaped macroduct present between median lobes; plates or gland spines in space between median and second lobes with at least two apical fimbriations, usually more; with conspicuous ear-like lobes on body margin laterad of mouthparts.

The differences between *P. ziziphi* and others species of *Parlatoria* present on *Citrus* spp. in Colombia are given in the key.

Material studied

Parlatoria ziziphi (Lucas). Colombia, Atlántico, Sabanagrande, Vda Los Caracoles, 9 m of altitude, 10°47'16.3" N, 74°46'07.2" W, 8th december 2015, coll. O. Dix; E. Palacino ex. *Citrus x limonia* (Rutaceae), 23♀♀, Catal. UNAB 1885.

Updated list of species of Diaspididae on *Citrus* spp. from Colombia. Author(s) after the colon (:) correspond to whom recorded each species.

Acutaspis scutiformis (Cockerell, 1893): Figueroa (1977).
Aonidiella comperei (McKenzie, 1937): Present study.
Aspidiotus nerii (Costa, 1829): Gallego and Vélez (1992), Kondo *et al.* (2012), Posada (1989).
Aulacaspis tubercularis (Newstead, 1906): Kondo *et al.* (2012), Posada (1989).
Chrysomphalus aonidum (Linnaeus, 1758): Balachowsky (1959), Figueroa (1946, 1952, 1977), Gallego and Vélez (1992), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Chrysomphalus dictyospermi (Morgan, 1889): Figueroa (1946, 1952, 1977), Gallego and Vélez (1992), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Hemiberlesia lataniae (Signoret, 1869): Kondo *et al.* (2012), Posada (1989).
Hemiberlesia palmae (Cockerell, 1892): Kondo *et al.* (2012), Posada (1989).
Ischnaspis longirostris (Signoret, 1882): Balachowsky (1959), Gallego and Vélez (1992) Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Lepidosaphes beckii (Boisduval, 1868): Balachowsky (1959), Figueroa (1952, 1977), Gallego and Vélez (1992), Mosquera (1979), Kondo (2001), Kondo *et al.* (2012).
Lepidosaphes gloverii (Packard, 1869): Balachowsky (1959), Figueroa (1952, 1977), Kondo (2001), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Lopholeucaspis sp.: Mosquera (1979), Posada (1989).
Parlatoria cinerea (Hadden in Doane and Hadden, 1909): Kondo (2001), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Parlatoria pergandii (Comstock, 1881): Kondo (2001), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Parlatoria ziziphi (Lucas, 1853): Blackburn and Miller (1984)
Pinnaspis aspidistrae (Signoret, 1869): Figueroa (1952, 1977), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Pinnaspis strachani (Cooley, 1898): Balachowsky (1959), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Pseudaonidia trilobitiformis (Green, 1896): Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Selenaspidus articulatus (Morgan, 1889): Balachowsky (1959), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).
Unaspis citri (Comstock, 1881): Balachowsky (1959), Figueroa (1952, 1977), Gallego and Vélez (1992), Kondo (2001), Kondo *et al.* (2012), Mosquera (1979), Posada (1989).

Key to subfamilies, genera and species of Diaspididae on *Citrus* spp. from Colombia (Compiled from Beardsley (1966), Claps and Wolff (2003), Kosztarab (1996), McKenzie (1946), Miller (2005), Miller and Davidson (2005) and Watson (2016)).

1. Macroducts normally of the "one-barred type", length of each one at least six times the width; second pygidial lobes unilobulate; anterior spiracles usually without associated pores; antennae of adult female usually bearing only one seta; pygidial plates present in second instar if not in adult; gland spines absent; duct tubercles absent; body circular or pyriform (Figure. 4A) .... **Aspidiotinae** 12
1'. Macroducts of the "two barred" type, length of each one rarely longer than three times their width; second pygidial lobes uni-or bilobulate; anterior spiracles usually with associated pores; antennae of adult female often bearing more than one seta; pygidial plates or gland spines often present in second instar if not in the adult; gland spines present or absent; duct tubercles may be present; body usually elongate

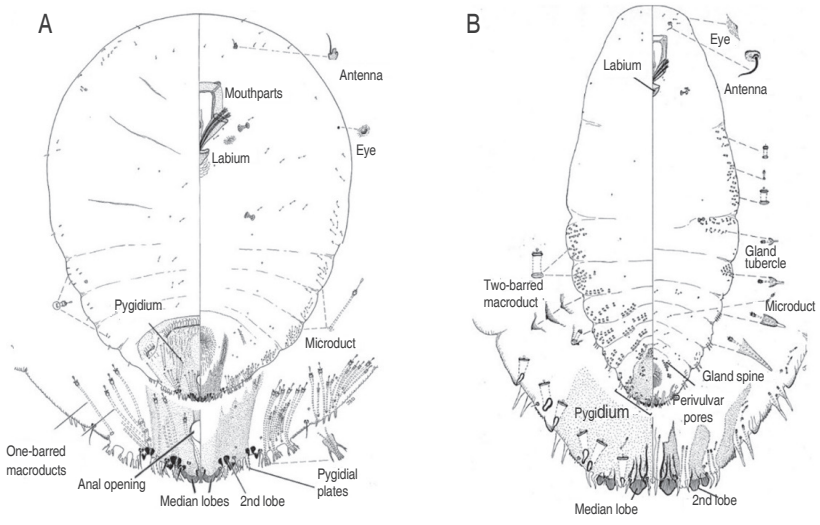

Figure 4. Habitus with magnified structures of two main subfamilies of Diaspididae. A. Aspidiotinae (Illustration from Miller and Davidson 1998); B. Diaspidinae. Illustration by Davidson (Miller, 2005).

spindle-shaped, rarely pyriform (Figure 4B) ..... **Diaspidinae** 2

2. Adult female with second lobes bilobulate (Figure 5A); gland spines usually present, occasionally replaced by plates; pores often associated with anterior and posterior spiracles; marginal macroducts usually with the long axis of each orifice perpendicular to margin; antennae each usually with two or more setae ……..……..……..…….. 6

2'.Adult female with second lobes unilobulate (Figure 5B); gland spines absent, plates usually present; spiracular pores associated with anterior spiracles only; marginal macroducts often with the long axis of each orifice oriented parallel to margin; antenna with one or more setae 3

3. Antenna with two to six setae; adult female pupillarial; body more or less elongate; plates, if present, confined to pygidium; marginal pygidial ducts not enlarged; abdominal disc pores sometimes present on some prepygidial segments ….....…… ***Lopholeucaspis* sp.**

3' Antenna with one seta; adult female usually not pupillarial; body oval to circular (elongate if pupillarial); plates often

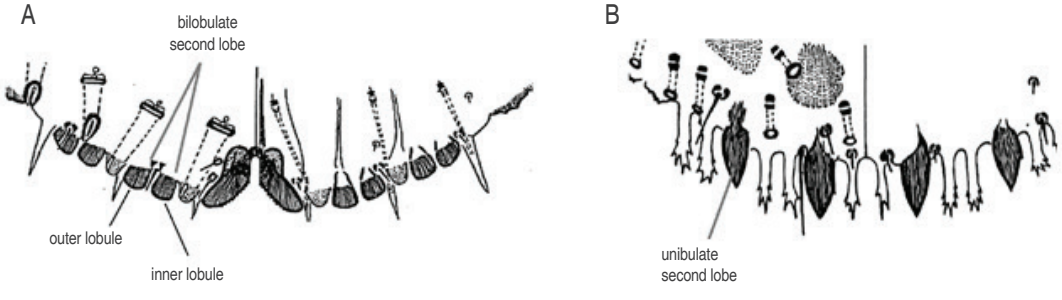

Figure 5. A. Pigydium with second lobe bilobulate; B. Pigydium with second lobe unilobulate. Illustrations taken from Miller and Davidson (2005).

present on pre pygidium and pygidium; marginal pygidial ducts often enlarged; abdominal disc pores never present on some prepygidial segments…….....…. ***Parlatoria*** (Targioni Tozzetti) 4

4. With a large, conspicuous, lateral and membranous lobe on each side of head, marginal to each anterior spiracle; female scale composed principally of a large black second exuvium ***..... Parlatoria ziziphi*** (Lucas) (Figure 6).

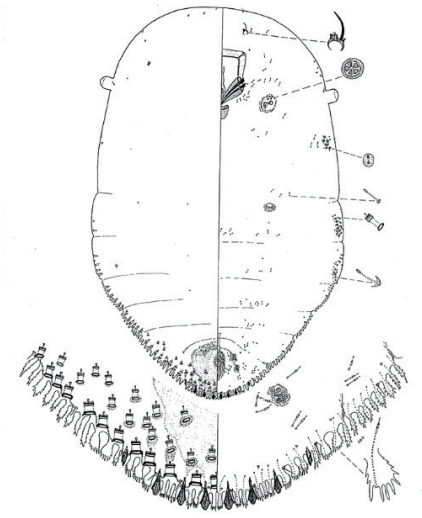

Figure 6. Habitus of *Parlatoria ziziphi* (Lucas) with magnified structures. Illustration taken from Miller and Davidson (2005).

4' Without such a membranous lobe on each side of head; female scale cover not largely black .....…5
5. Dorsum of pygidium with two longitudinal rows of macroducts on each side of pygidium on abdominal segments six and seven, extending cephalad to well anterior of anal opening; three plates between third and fourth lobs ..... ***Parlatoria cinerea*** (Hadden in Doane and Hadden) (Figure 7A)
5'. Dorsum of pygidium without such rows of macroducts; macroducts not found anterior to anal opening on these segments; four plates between third and fourth lobes ..... ***Parlatoria pergandii*** (Comstock) (Figure 7B)
6. Median lobes zygotic (Figure 8A) ….....…… 7.
6'. Median lobes not zygotic (although crowding or secondary sclerotization may make this difficult to see) (Figure 8B) 9
7. Gland spines and macroducts absent from area anterior to abdominal segment 1; body of characteristic shape, with head, prothorax, and mesothorax swollen and rectangular; lateral margin side of prothorax on older females with 1

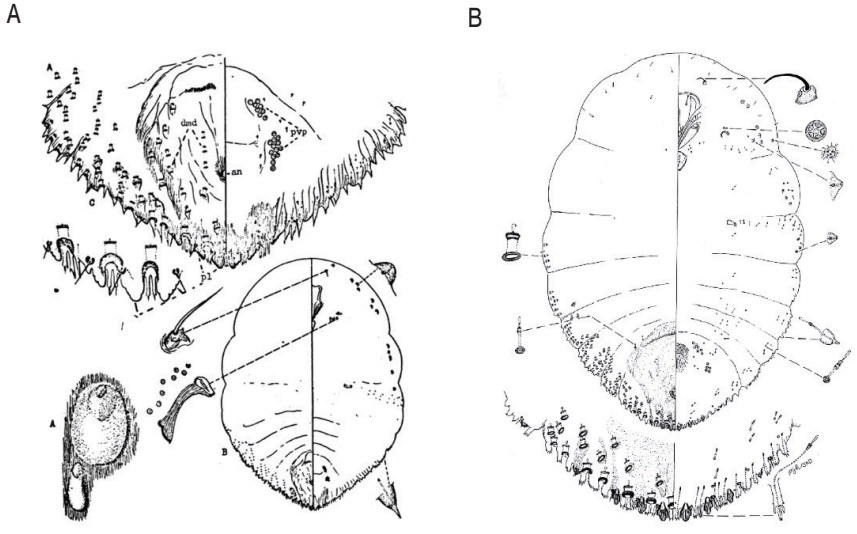

Figure 7. Habitus with magnified structures of: A**.** *Parlatoria cinerea* (Hadden in Doane & Hadden) (Illustration taken from Gerson 1977); B. *Parlatoria pergandii* (Comstock). Illustration taken from Miller and Davidson (2005).

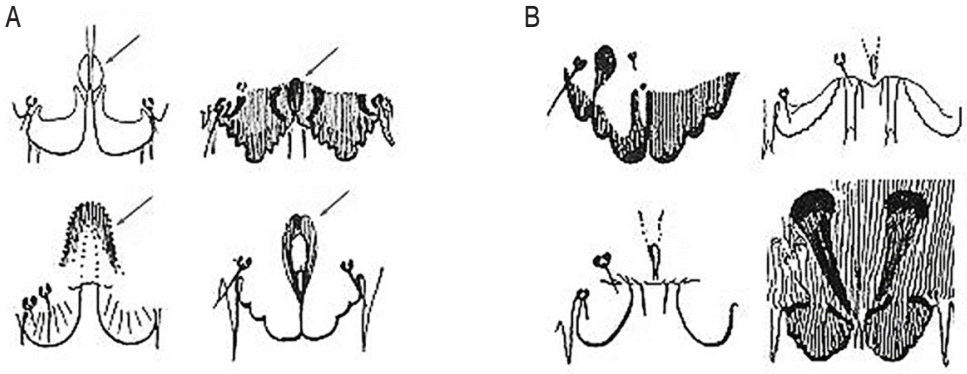

Figure 8. A. Median lobes zygotic; B. Median lobes not zygotic. Illustrations taken from Watson (2016).

swollen tubercle on each side of body; labium set in groove, with sclerotized areas on each side…….. ***Aulacaspis tubercularis*** (Newstead) (Figure 9).

7'.Gland spines and macroducts present anterior to abdominal segment 1; head, prothorax, and mesothorax not swollen; without lateral tubercles on lateral margin side of prothorax on older females; labium set not in groove, without sclerotized areas on each side…....……***Pinnaspis*** (Cockerell).....8

8. Preanal sclerosis lacking or represented by light sclerotized patch; median lobes protrude less than or about same distance as second lobes; posterior spiracles each

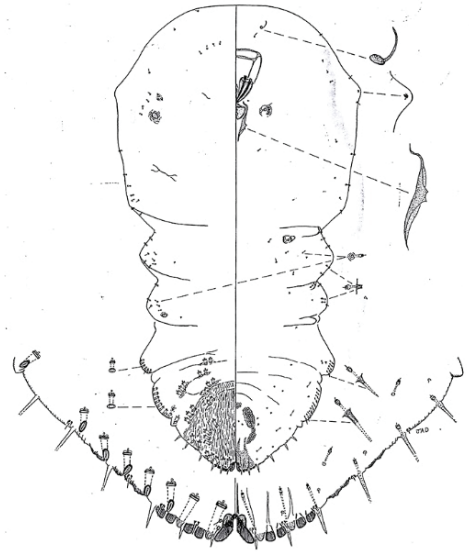

Figure 9. Habitus with magnified structures of *Aulacaspis tubercularis* (Newstead). Illustration taken from Miller and Davidson (2005).

with one to 12 pores, four pores on average ……..…… ***Pinnaspis aspidistrae*** (Signoret) (Figure 10A)

8'. Preanal sclerosis represented by sclerotized bar; median lobes protrude beyond or about same distance as second lobes; posterior spiracles each with 0 to four pores, two pores on average …….…….. ***Pinnaspis strachani*** (Cooley) (Figure 10B)

9. Median lobes with only a pair of marginal setae between their bases ……..……11

9'. Median lobes with only a pair of gland spines between their bases …..…… ***Lepidosaphes*** (Schlectendal) 10

10. Mature female without sclerotized pattern on thorax; without sclerotized dermal pockets on thorax; macroducts

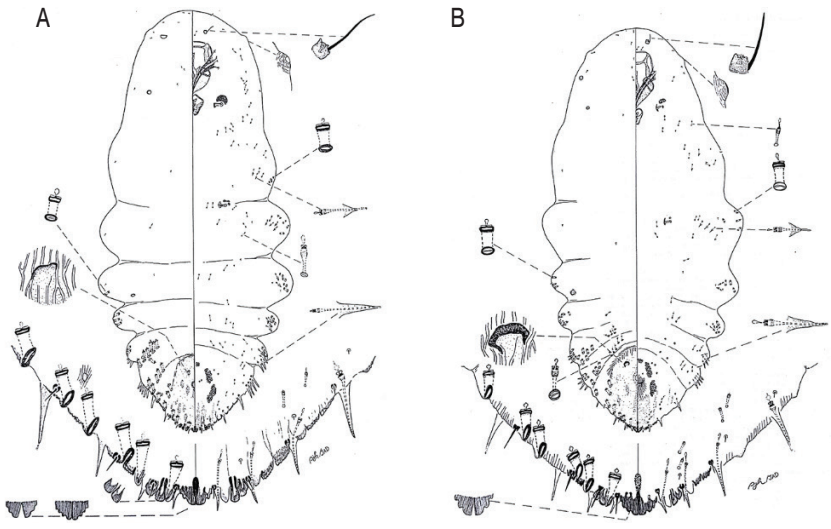

Figure 10. Habitus with magnified structures of: A. *Pinnaspis aspidistrae* (Signoret) B. *Pinnaspis strachani* (Cooley). Illustrations taken from Miller and Davidson (2005).

on dorsal margin of prothorax present ……***Lepidosaphes beckii*** (Boisduval) (Figure 11A)

10'. Mature female with distinctive pattern of punctures on dorsum of thorax extending to ventral margin; with sclerotized dermal pockets on pro- and mesothorax; macroducts on dorsal margin of prothorax absent …….. ..... ……. ***Lepidosaphes gloverii*** (Packard) (Figure 11B)

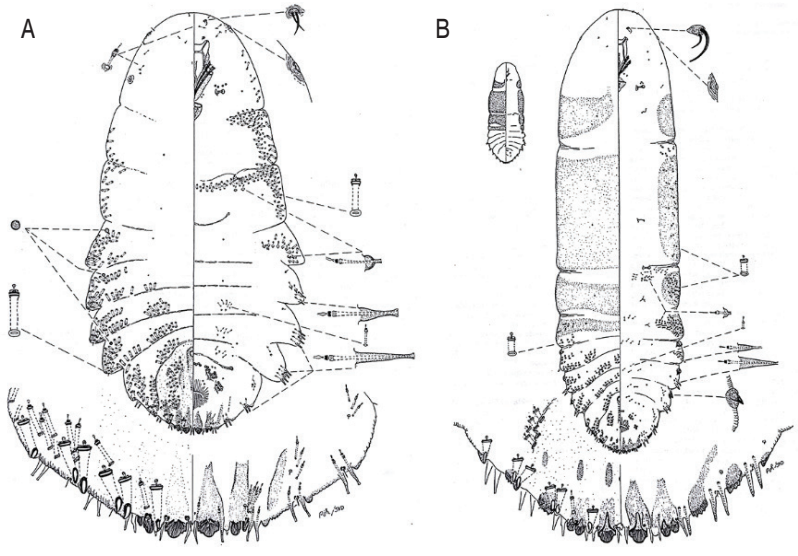

Figure 11. Habitus with magnified structures of: A. Lepid*osaphes beckii* (Boisduval); B. *Lepidosaphes gloverii* (Packard). Illustrations taken from Miller and Davidson (2005).

11. Dorsum of pygidium with a coarse 'lattice-work' sclerotized pattern ..... ***Ischnaspis longirostris*** (Signoret) (Figure 12A)

11'. Dorsum of pygidium without such a sclerotized pattern; with four or fewer perivulvar pores on each side of body ..... ***Unaspis citri*** (Comstock) (Figure 12B)

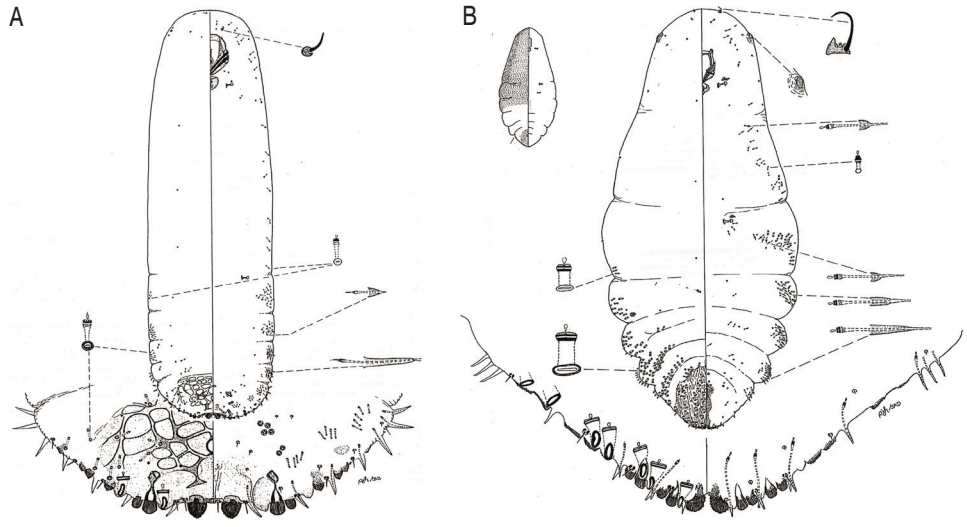

Figure 12. Habitus with magnified structures of: A. *Ischnaspis longirostris* (Signoret); B. *Unaspis citri* (Comstock). Illustrations taken from Miller and Davidson (2005).

12. Dorsal surface of pygidium with a conspicuous mosaic or areolate appearance ……. ***Pseudaonidia trilobitiformis*** (Green) (Figure 13A)

12'. Dorsal surface of pygidium without mosaic areolate pattern …… 13

13. Pygidial margin with paraphyses present, these sometimes small (arising from bases of lobes or from the margin itself); paraphyses sometimes present lateral to the outer lobes ……14

13'. Pygidial margin without paraphyses ……. 19

14. Pygidial apex acute, forming an angle of 90° or less, although basal half of pygidium may be broad (Figure 13B); pygidial margins tending to be concave and sclerotized; plates confined to interlobular spaces …… ***Acutaspis scutiformis*** (Cockerell)

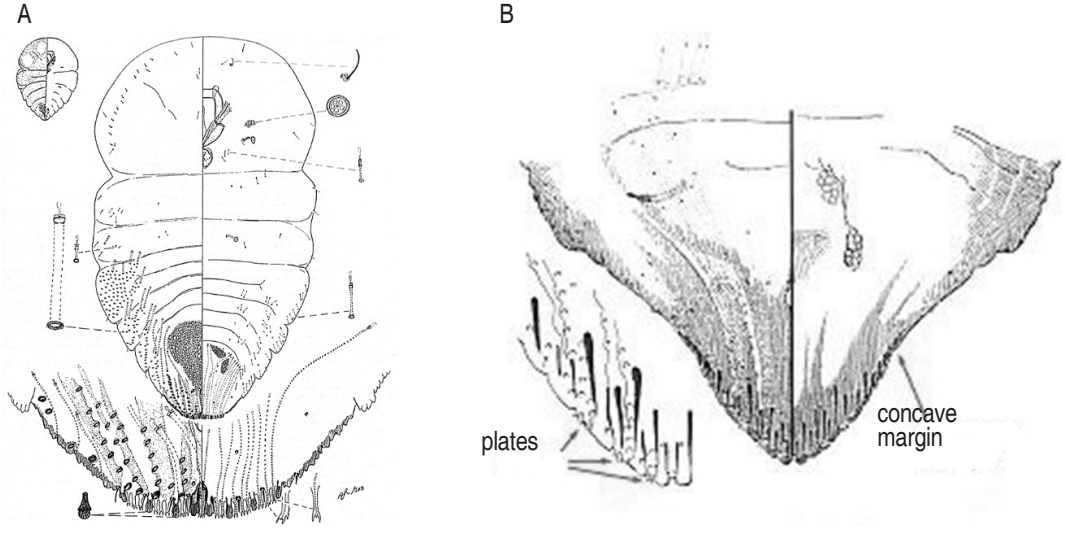

Figure 13. A. Habitus with magnified structures of *Pseudaonidia trilobitiformis* (Green). Illustration from Miller and Davidson (2005); B. Pygidial apex acute, forming an angle of 90° or less of *Acutaspis scutiformis* (Cockerell). Illustration taken from Watson (2016).

14'. Pygidial apex more or less broad, forming an angle greater than 90°; pygidial margins convex or straight, with or without sclerotization; plates often present lateral to third lobes ……..…….. 15

15. Mature specimens with prosoma very enlarged and sclerotized, usually reniform so its lateral lobes more or less enclose the pygidium. If prosoma not reniform at maturity (*A. orientalis*), then paraphyses between second and third lobes never longer than the lobes.....……. ***..... Aonidiella comperei*** McKenzie (Figure 14)

15'. Mature specimens with prosoma membranous or sclerotized, not expanded sufficiently to become reniform; paraphyses between second and third lobes shorter or longer than the lobes ……..……..... 16

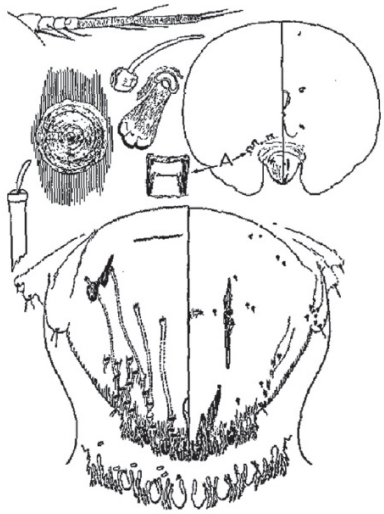

Figure 14. Habitus with magnified structures of *Aonidiella comperei* McKenzie. Illustration taken from McKenzie (1937).

16. Paraphyses obviously longer than median lobes (Figure 15A) …… ***Chrysomphalus*** (Ashmead)…..17

16' Paraphyses same length or shorter than median lobes (Figure 15B) ……..……..…… ***Hemiberlesia*** (Berlese and Leonardi) .....….....18

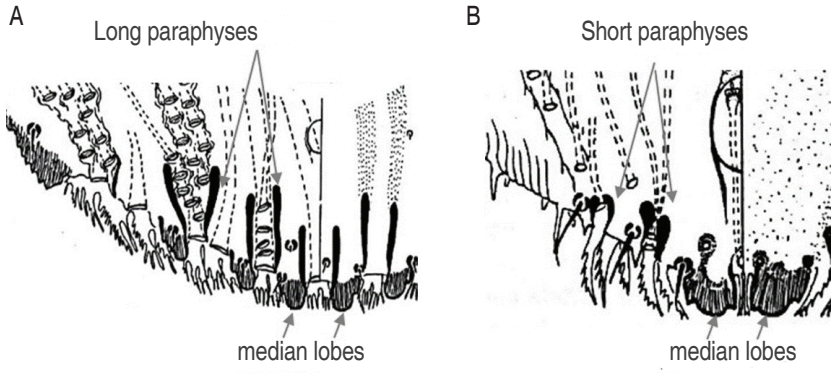

Figure 15. A. Paraphyses obviously longer than median lobes; B. Paraphyses same length or shorter than median lobes. Illustrations taken from Miller and Davidson (2005).

17. With one cluster of macroducts on submarginal areas of prepygidial segments; plates just lateral to third lobes fringed; nine to 13 prevulvar pores on each side of pygidium ……. ***..... Chrysomphalus aonidum*** (Linnaeus) (Figure 16A)

17'. Without clusters of macroducts on submarginal areas of prepygidial segments; plates just lateral to third lobes clavate; five or six prevulvar pores on each side of pygidium ..... ***Chrysomphalus dictyospermi*** (Morgan) (Figure 16B).

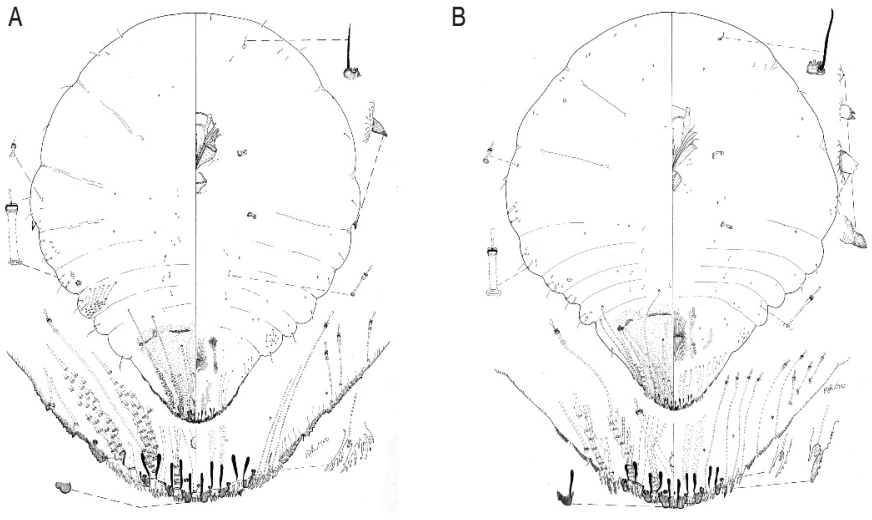

Figure 16. Habitus with magnified structures of: A. *Chrysomphalus aonidum* (Linnaeus); B. *Chrysomphalus dictyospermi* (Morgan). Illustrations taken from Miller and Davidson (2005).

18 Medial notch, first and second space of pygidium with band of fringe plates less strongly developed; plates in third space, if present, reduced in size, not forming such a dense uniform fringe; median lobes very close together, almost in touch; ..... ***Hemiberlesia lataniae*** (Signoret) (Figure 17a)

18'. Medial notch, first, second and third space of pygidium with a band of broad apically fringed plates of nearly uniform; plates in third space always present in a dense, nearly uniform fringe; median lobes separated, at least in the half of the width of each median lobe ..... ***Hemiberlesia palmae*** (Cockerell) (Figure 17b)

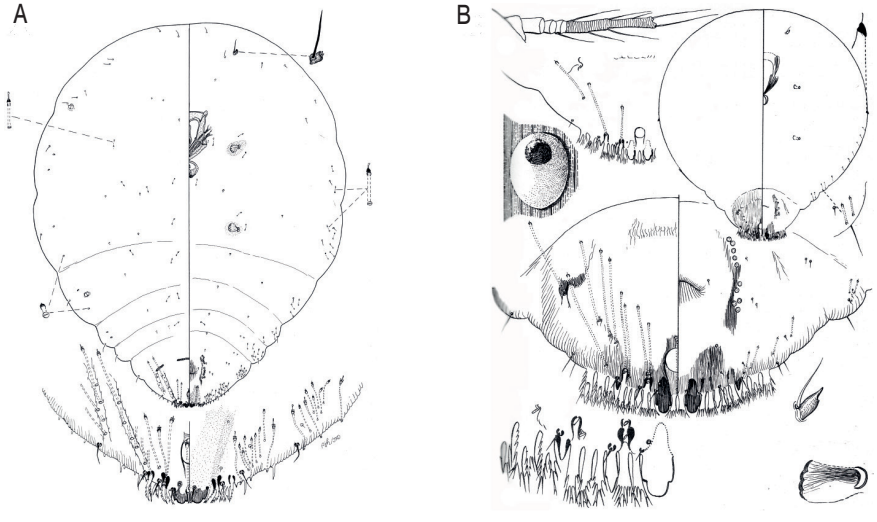

Figure 17. Habitus with magnified structures of A. *Hemiberlesia lataniae* (Signoret). Illustrations taken from Miller and Davidson (2005); B. *Hemiberlesia palmae* (Cockerell). Illustrations taken from Ferris (1938).

19. Prosoma strongly constricted between meso- and metathorax, and sclerotized; third lobe represented by an elongate, acute, sclerotized spine.....…….....…… .....***Selenaspidus articulatus*** (Morgan) (Figure 18a)

19'. Prosoma without strong constriction between meso- and metathorax, and usually membranous; third lobe short, either rounded or pointed, or absent…….. ……..... ***Aspidiotus nerii*** (Costa) (Figure 18b)

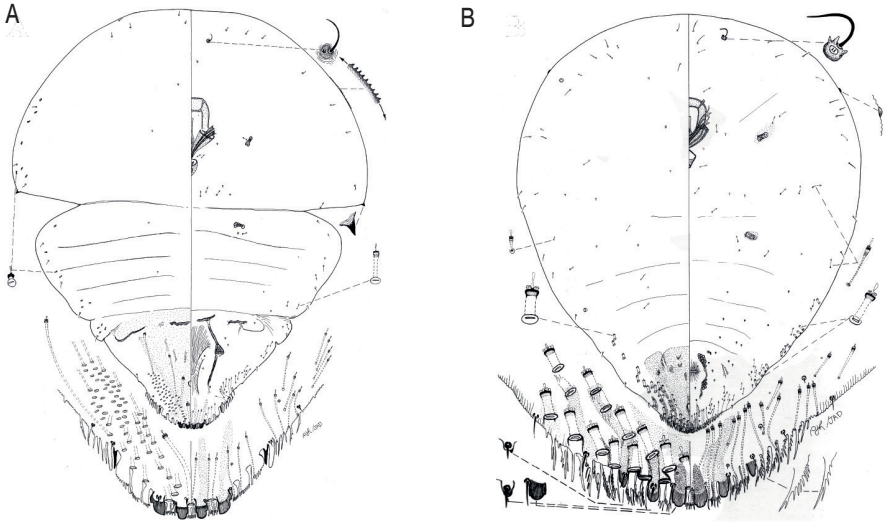

Figure 18. Habitus with magnified structures of: A. *Selenaspidus articulatus* (Morgan); B. *Aspidiotus nerii* (Costa). Illustrations taken from Miller and Davidson (2005).

CONCLUSIONS

The identity of *Aonidella comperei*, the species that is causing damage on fruits, branches and leaves of *Citrus aurantifolia* in Tolima, Colombia, was determined. This basic information will be useful to start studies about its biology and ecology in order to define management strategies. The knowledge about species of Diaspididae on *Citrus* spp. in Colombia is improved and the number of species recorded on this host and their geographical distribution is updated.

ACKNOWLEDGEMENTS

We thank to William King by the information and the logistic to the collect the samples of *Aonidiella comperei* and Oscar Dix for providing samples of *Parlatoria ziziphi*. Thanks to Lucia Claps and Barbara Denno for providing scientific literature. Thanks to Museo Entomológico UNAB and Instituto Colombiano Agropecuario Ica-Ceisa by the facilities to develop this research. Thanks to Takumasa Kondo and anonymous reviewers for theirs comments and suggestions. Thanks to Douglas Miller, John Davidson and Stephanie Munson (Cornell University) by the permission to use pictures and images.

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Research article

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Revista Facultad Nacional de Agronomía

Characterization of soils cultivated with rubber in the Colombian Bajo Cauca Antioqueño region

UNIVERSIDAD NACIONAL DE COLOMBIA

Caracterización de suelos cultivados con caucho en el Bajo Cauca Antioqueño, Colombia

doi: 10.15446/rfna.v70n2.64520

Martin Rodrigo Villa Cuastumal[1]*, Enrique Guillermo Martínez Bustamante[1], José Régulo Cartagena Valenzuela[1], Orlando Antonio Rodríguez Rodríguez[2] and Nelson Walter Osorio[3]

Key words:
Hevea brasiliensis
Ultisol
Plant nutrition
Colombian soils

ABSTRACT

From a socioeconomic and environmental standpoint, rubber cultivation is potentially important for Colombia, because it not only constitutes an alternative to the traditional mining, agricultural, and livestock activities, but also a beneficial option in the substitution of illegal crops. Thus, there is need to determine the factors restricting the productivity of the species, particularly soil-related aspects. In this context, the current research undertook the description and identification of soil physical and chemical properties in three rubber plantations located in a region known as *Bajo Cauca Antioqueño*, at the lower course of the Cauca river. The results indicate that these soils are classified as Ultisols, they are deep, with acid reaction and low levels of soil organic matter. Essential elements also exhibited low levels, except Cu. Fe and Al are also present in very high concentrations, while the cation exchange capacity is limited. These observations alert on the need for agronomic management techniques that allow the crop to express its full potential under conditions that are not very adequate for its growth and development.

Palabras claves:
Hevea brasiliensis
Ultisol
Nutrición de plantas
Suelos colombianos

RESUMEN

El cultivo del caucho es un rubro agrícola de importancia ambiental y socioeconómica para Colombia, por ser una alternativa diferente de las actividades mineras y agropecuarias tradicionales; incluso, representa una opción beneficiosa para la sustitución de cultivos de uso ilícito. Lo anterior muestra la necesidad de determinar los factores que restringen su producción y la productividad de la especie, con énfasis en lo relacionado al recurso edáfico. En ese sentido, se hizo la descripción y la identificación de las propiedades físicas y químicas del suelo de tres predios ubicados en el *Bajo Cauca Antioqueño* dedicados a la explotación de la euforbiácea. Los resultados indicaron que los suelos son del orden Ultisol, profundos, de reacción ácida y con contenido bajo de materia orgánica; los elementos esenciales están en niveles bajos, excepto el Cu. El Fe y el Al se encuentran en cantidades muy altas, mientras que la capacidad de intercambio catiónico es limitada. Lo observado alerta sobre la necesidad de aplicar técnicas de manejo agronómico que permitan que esta especie exprese todo su potencial en condiciones que son poco aptas para su crecimiento y desarrollo.

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. AA 1779, Medellín, Colombia.
[2] Decanato de Agronomía. Universidad Centrooccidental Lisandro Alvarado. Apartado Postal 400. Barquisimeto, Venezuela.
[3] Facultad de Ciencias. Universidad Nacional de Colombia. AA 3840. Medellín, Colombia.
* Corresponding author: <mrvillac@unal.edu.co>

Received: September 29, 2016; Accepted: April 4, 2017
Rev.Fac.Nac.Agron. 70(2): 8155-8167. 2017
ISSN 0304-2847 / e-ISSN 2248-7026

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The rubber [*Hevea brasiliensis* (Willd. Ex A. Juss.) Müll. Arg.] cultivated area in Colombia is currently growing, with an average annual increase of 3928 ha for the period between 2002 and 2008, and a total area of 45,000 ha in 2014 (Castellanos *et al*., 2009). However, there is still a deficit of 12,000 yearly tons that are needed to supply the 17,000 yearly tons demanded by the domestic industry (Castiblanco, 2014). That is, the country is not self-sufficient, since it imports 70% of its domestic rubber consumption. A similar trend can be observed at the world level, where a rubber deficit of 170,000 to 200,000 tons is projected for the period between 2005 and 2020 (Santacruz, 2008).

The current increase in the domestic and global natural rubber demand makes the cultivation of rubber an important segment of the national cropping activity. The demand comes from the leather, automobile,and chemical industries (67%) and from the manufacture of latex-made products (11%, corresponding to, *e.g.*, surgical, household and industrial gloves); other products such as conveyor belts, hoses, gaskets, footwear, adhesives and others represent 22% (Castellanos *et al*., 2009; SADRA, 2011).

From a socio-economic standpoint, rubber cultivation constitutes an important cropping activity for Colombia, because it provides an alternative to the traditional mining, agricultural and livestock activities. Furthermore, it represents an alternative in the replacement of illegal-crops, by generating one direct job and three indirect jobs every four ha of the crop (STNCN, 2008; Castellanos *et al.,* 2009; SADRA, 2011).

According to UPRA (2015), Colombia has 18 144 457 ha with adequate conditions for rubber cultivation; but only 18.7% of them, have the best conditions from the physical, socio-ecosystem and socio-economic point of view. The remaining 81.3% are located in areas characterized by low to average aptitude for the crop, with moderate to severe limitations for its development. In the department of Antioquia, the situation is very similar: out of 1 358 079 ha that are suitable for rubber cultivation, 13.6% exhibit optimal conditions; 48.3% present average conditions (predominantly those in the region of Urabá); while those exhibiting low aptitude, which concentrate in the Bajo Cauca Antioqueño (*BCA*) region, account for 38.1% of the total area. At the departmental level, the edaphic characterization, will allow to locate the soils with better aptitude for use.

Despite the above restrictions, Santacruz (2008), Castellanos *et al.* (2009) and SADRA (2011) have found that the growing demand for natural rubber at the local and global levels is attracting not only for private investors (rubber growing IRR is 16 to 18%), but also for the national government, who are determined to strengthen the sector. This situation constitutes an excellent opportunity for Colombia in the sense of becoming self-sufficient in the medium and long term, and even opens the possibility to move from rubber raw material imports to exports.

To achieve this goal, it is necessary to overcome existing technological constraints such as poor agricultural management of plantations (particularly regarding nutrition and the handling of biotic and abiotic factors), scarce use of production and post-production technologies and innovations, little information on latex agroindustrial management plans, and limited research at the regional level. Consequently, it is important to strengthen research, technical assistance and technology transfer in order to develop and improve the quality of the material thus produced. Also, it is necessary to optimize the post-production processes and provide farmers with adequate training. The comprehensive articulation of these measures is likely to improve quality, overcome both low yields and national production deficit, and achieve exportable surpluses.

Antioquia has 4098 ha of rubber plantations, 13.5% of which yield 1.2 t ha^{-1} $year^{-1}$, which is within the world average range (0.9 to 1.3 t ha^{-1} $year^{-1}$), but is low if compared with the yields of India and Thailand (1.9 and 1.8 t ha^{-1} $year^{-1}$, respectively) (Santacruz, 2008; Castellanos *et al.,* 2009). Another potential benefit derived from the establishment of the species in this country is highlighted by SADRA (2011), who state that by the year 2020 this crop will be favoring the life conditions of 6000 families in the Colombian rubber production strip, which covers the departments of Antioquia and Córdoba. Moreover, this crop exerts a positive impact on the environment through carbon (CO_2) fixation, oxygen release (O_2), and other ecosystem services.

Adequate climatic conditions for the cultivation of rubber in the country correspond to areas with annual rainfall

between 2000 and 4000 mm, temperature above 24 °C, and sunshine higher than 1700 h year^{-1}. However, plantations can be established in regions with medium to low aptitude for the crop, wherein precipitation can be either below or above the optimal range (1000 to 2000 and 4000 to 5000 mm year^{-1}, respectively), temperatures between 18 and 24 °C, and sunshine from 900 to 1700 h year^{-1} (UPRA, 2015).

Compagnon (1998) considers rubber as undemanding crop with regards to soil characteristics. Having evolved mainly on tropical soils (Martínez, 2007), rubber is highly adaptable to the different climate and soil conditions of the tropics, including extremely low fertility levels.

The necessary soil texture conditions for the proper development of the root system of a rubber tree should be loam to silty loam, appropriate structure, and effective depth >150 cm, which facilitate good moisture retention (Escobar *et al.,* 2004; SENA, 2006).

With respect to pH, the species grows well in a range of 4.5 to 5.5. Although, elevated pH levels are not recommendable (Martínez, 2007), it is clear that at lower levels of pH bases become scarce and use of amendments (e.g., lime and gypsum) is needed, resulting thus in increased production costs. Satisfactory organic matter (OM) content in the first 20 cm soil layer is 3.0 - 4.0%, which guarantees C and N contents of 20 000 and 2000 kg ha^{-1}, respectively. In this way, when the C/N ratio ranges between 10 and 12, N release is favored (Umoh *et al*., 2014).

Regarding the Effective Cation Exchange Capacity (ECEC), Torres (1999) reports that well developed plantations where found when this parameter did not exceed $cmol_c$ kg^{-1}. In addition, this author states that rubber can be planted on flat ground (which is important from the standpoint of mechanization) or on soils with slopes below 15%, following contour lines. In both cases there have been satisfactory latex yields of 1.4 t ha^{-1} year^{-1}.

Santana and Díaz (1999) mention that some of the soil chemical characteristics in the BCA region favor the development of rubber plantations, although there are contrasting variations from one place to another, mainly determined by topography and soil management differences.

Based on appropriate soil nutrient content standards for rubber cultivation, it can be anticipated that low levels of B (<0.6 mg kg^{-1}), Ca (<3.0 $cmol_c$ kg^{-1}), P (<15 mg kg^{-1}) and K (<0.15 $cmol_c$ kg^{-1}) (Azabache, 2012), as well as high levels of Fe (>50 mg kg^{-1}), hinder the proper development of rubber plantations in the BCA region soils (Oku *et al.,* 2012). Moreover, the low OM content of these soils (<1.5%) is likely to constrain the proper development of the crop (Torres, 1999).

From a pedogenetic point of view, Osman (2013) indicates the climate as the fundamental factor in the process of soil formation, specifically recognizing rain and temperature regimes as the most influential factors. Similarly, Abreu Jr, *et al.* (2003) indicate that good part of the humid tropic soils exhibit high levels of nutrient leaching, coupled to low natural fertility. According to the mentioned author, this is the result of a combination of factors such as strongly to extremely soil acidity, presence of available and exchangeable Al, high P fixation capacity, low Ca, Mg and K levels, low cationic and high anionic exchange capacities, and elevated concentrations of some metallic micronutrients such as Fe, Cu, Mn and Zn.

In that way, the characterization of soil physical-chemical attributes is a key management tool in the improving of agronomic processes and the increasing of productivity and competitiveness of the rubber sector in the *BCA* region. Currently, there is no detailed knowledge of the rubber cultivated soils of the *BCA* region. The closest approach corresponds to the general study of soils and land cover of the Department of Antioquia, conducted in 2007 by the Instituto Geográfico Agustín Codazzi (IGAC) at scales 1:100 000 and 1:25 000.

All these considerations highlight the need to identify the limiting factors in the production and productivity of rubber plantations in the Antioquia department, Colombia, Latin America and other places in the world, primarily referring to the soil. In this context, and considering that adequate soil management and climate allow the species to express its genetic potential and increase its efficiency and competitiveness, the present research aimed to provide a general characterization and taxonomic identification

of the rubber-cultivated soils of the *BCA* region, and to determine their agronomic potential under the agro-ecological conditions of the region.

MATERIALS AND METHODS

Location

The characterization of the soils and their physical and chemical attributes was carried out in three representative rubber plantations of the *BCA* region. The first site corresponds to Villa Gina (7°43'1.4"N, 75°30'36.1"W; 131 m of altitude; 3133 mm of annual rainfall; and 27.0 °C of temperature), established in the locality of Santa Clara, within the limits of the municipality of Tarazá. This property belongs to the Rubber Growers Committee Association (Asociación Comité de Cultivadores de Caucho – ASCULTICAUCHO).

The second site corresponds to La Envidia (7°53'1.6"N, 74°50'14.2"W; 54 m of altitude; 3529 mm of annual rainfall; and 27 °C temperature), established in the locality of Quebrada La Ciénaga, within the limits of the municipality of Nechí. The farm belongs to the Rubber Growers Association of Cargueros and Bijagual – ASCABIA.

The third site was located in La Golondrina (7°52'36.7"N, 74°57'5.5"W; 77 m of altitude; 2575 mm of annual rainfall and 27.7 °C temperature established in the locality of Bella Palmira, within the limits of the municipality of Caucasia. The three farms are included in the tropical rain forest (rf-T) ecological life zone (Holdridge, 1987; Espinal, 1992).

Soil description

In each of the rubber plantations, a one-piece combination Edelman auger was employed to take soil samples at seven plots representing the topographical variations of the area. Based on horizon A depth contrasts, color assessed through Munsell table, texture estimated by touch, and structure estimated through aggregate size, one of the sampled plots at each farm was selected to dig a pit of 1.5 m long x 1.2 m wide x 1.5 m deep. Characteristics such as soil texture, color, structure, temperature, root distribution, mottling, consistency, pore size, and amount, presence of macroorganisms, anthropogenic (mining) activity, signals of compaction, and horizon thickness were described *in situ*, according to methodologies by USDA (1993), Jaramillo (2002), IGAC (2007 b) and FAO (2009).

Additionally, the following tests were performed in each soil horizon: (a) reaction to hydrogen peroxide (H_2O_2), in order to indicate the presence of decaying OM and/or Mn; (b) reaction to 4% sodium fluoride (NaF) and phenolphthalein impregnated filter paper to identify the presence of allophanes or soil with andic conditions; and (c) reaction to 10% hydrochloric acid (HCl), to determine the presence of free carbonates. All these evaluations followed guidelines of USDA (1993) and Jaramillo (2002).

Field information was contrasted to soil maps prepared by IGAC (2007a) for the municipalities of Tarazá, Nechí, and Caucasia, the guidelines of the American Soil Taxonomy (USDA, 2006), and the taxonomic system established by FAO (2006). This allowed the classification and morphological, physical, and chemical characterization of the areas of interest.

Determination of soil physical properties

Soil resistance to penetration (MPa) assessment were carried out *in situ* from 0 to 0.30 m deep, making use of a compaction meter (FieldScout SC900®). Soil moisture (%) measurement was conducted from 0 to 0.90 m deep, using a FieldScout TDR 300 soil moisture meter. For the construction of moisture retention, porosity (%), bulk density (g cm^{-3}) and hydraulic conductivity (cm min^{-1}) curves, undisturbed samples were taken from the first 0.25 m of the soil profile in PVC cylinders of 0.0762 m diameter and 0.10 m high. The soil columns thus obtained were taken to the laboratory in closed metal boxes following ASTM norm D4220 (2000). The analysis of these samples was conducted in the laboratory of Soil Physics and Conservation of the Universidad Nacional de Colombia at the Medellin *campus*, following protocols by IGAC (1990), USDA (1993), and Jaramillo (2002).

Soil fertility assessment

Soil samples for chemical analysis were taken from the first 0.25 m of the soil profile with a one-piece combination Edelman auger. Twenty-five to 30 subsamples made up a composite sample following guidelines by IGAC

(1990), Carrillo *et al.* (1995) and Gómez (2005). Chemical determinations were carried out at the Soil Laboratory of the Universidad Nacional de Colombia, Medellín *campus*. The methods employed were: texture by Bouyoucos; pH in water 1:1, weight/volume; Organic Matter content (%) and organic C (%) by Walkey and Black; P (mg kg^{-1}) by Bray II and colorimetric method; K, Ca, Mg ($cmol_c$ kg^{-1}) by ammonium acetate at pH 7.0 and atomic absorption; Fe, Mn, Cu, Zn (mg kg^{-1}) by Olsen (0.5 M $NaHCO_3$ and EDTA) and atomic absorption; B (mg kg^{-1}) extracted by hot water; Al ($cmol_c$ kg^{-1}) by 1 M KCl and atomic absorption; S (mg kg^{-1}) by 0.008 M monocalcium phosphate; NO_3^- (mg kg^{-1}) by 0.025 M aluminum sulphate; NH_4 (mg kg^{-1}) (1 *M* KCl); ECEC ($cmol_c \cdot kg^{-1}$) sum of exchangeable cations. Details about the methods can be seen at IGAC (2006).

RESULTS AND DISCUSSION

Soil description

Table 1 shows the characteristics of the three studied soils, which are representative of one of the major landscape units of Antioquia, namely the plains and low hills of the Cauca river basin. Resulting from ancient alluvial deposits brought by the tributaries of the Cauca river (Arbaux, 2003), this is one of the four main Colombian landscape types (IGAC, 1995). The characteristics observed at the sites of interest are the product of intimate soil-landscape correlation, wherein the stability of the geological formations and their constituent materials contribute to the evolution of the soils (Malagón, 2003) and represent the spatial and visual expression of the environment (Muñoz-Pedreros, 2004). This physiography is consistent with the views of Jaramillo (1996), who refers to the presence of three contrasting terraces in the region: a hilly and strongly dissected (upper) one, a number of flat or little dissected (medium and low) ones. These morphological differences are associated to different degrees of evolution. The warm and humid climate of the evaluated territory is described by Betancur *et al.* (2009) as particularly complex, due to the influence of the cyclonic curvature of the Caribbean waves, the proximity to the slopes of the western mountain range, and the effect of mid - latitude troughs.

Table 1. Relief diversity in three rubber planted areas of the lower basin of the Cauca River (Antioquia, Colombia).

Characteristic	Villa Gina (Tarazá)	La Envidia (Nechí)	La Golondrina (Caucasia)
Landscape	Flatland	Hillside	Terrace
Topography	Slightly undulating	Undulating	Slightly undulating
Slope	Slightly steep	Moderately steep	Slightly steep
Erosion type	No	Hydric: laminar Physical: crusting	Water: laminar
Degree of erosion	No	Low	Low
Climate	Warm wet	Warm wet	Warm wet
Type and class of surface stoniness	No	No	No
Water table (depth)	Not found	Not found	Not found
Frequency and duration of floods	None	None	None
Soil depth	Very deep	Very deep	Very deep
Effective depth	Moderately deep	Superficial	Moderately deep
Soil moisture regime	Udic	Udic	Udic
Soil temperature regime	Isohyperthermic	Isohyperthermic	Isohyperthermic
Soil moisture condition	Slightly wet	Moderately damp	Slightly wet
Edaphic climate	Udic isohyperthermic	Udic isohyperthermic	Udic isohyperthermic
Ground cover	Herbaceous	Herbaceous	Herbaceous
Cover height (cm)	30 - 35	35 - 40	30 - 35
Current usage	*Cecropia peltata* L. (4-5 m) and grasses	Grassland	*Brachiaria humidicola* (Rendle) Schweickerdt
Diagnostic horizons	Epipedon: ochric Endopedon: argillic	Epipedon: ochric Endopedon: argillic	Epipedon: ochric Endopedon: argillic

The profiles shown in Figure 1 and explained in Tables 2, 3, and 4 allow identifying the soils under study as Ultisols that resulted from sedimentary materials in hot and humid climates. These soils usually present acid reaction and exhibit red and yellow colors resulting from the accumulation of iron oxide (Oballos and Ochoa, 2008; Sánchez and Rubiano, 2015). In the current study, they generally exhibited good drainage, the dominant textures are silty loam (Villa Gina and La Golondrina) and clay loam (La Envidia). Effective depth was found to be variable, the soils of Villa Gina standing out for depths greater than 0.60 m, below which rubber trees usually develop most of their roots (Azabache, 2012). In the other two rubber plantations, the effective depth is considered to be sub-optimal, which implies certain restrictions that do not prevent the establishment of rubber plantations. The soils of La Envidia stand out for the vulnerability of their aggregates to external dispersing agents such as water, wind or mechanical manipulation (Pérez, 1992).

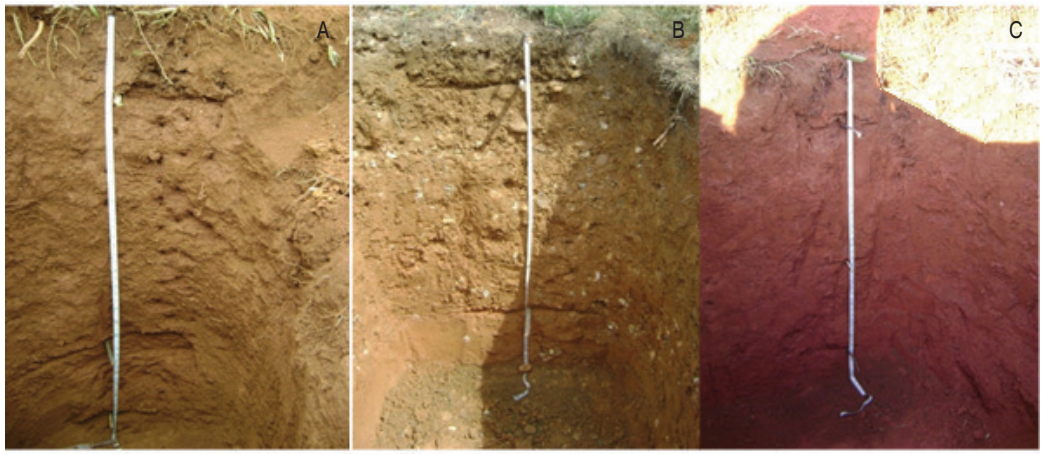

Figure 1. Typical soil profiles in three rubber planted farms of the Colombian Bajo Cauca Antioqueño region. A. Villa Gina (Tarazá); B. La Envidia (Nechí); C. La Golondrina (Caucasia).

Table 2. Soil description of *Villa Gina* rubber plantation (Tarazá).

Diagnostic horizons / depth	Description
Ap 00 - 20 cm	Red color when moist (2.5YR 4/6); silty loam texture; fine and very fine, moderately blocky sub-angular structure; slightly plastic and sticky consistency when moist; many fine and very fine pores; many fine and very fine, superficially distributed roots; presence of macroorganisms; strongly acid pH (4.5); violently effervescent reaction to decaying OM (H_2O_2); negative reaction to both free carbonates (HCl) and allophanes (NaF); clear and smooth boundary.
Bt 20 – 98 cm	Red when moist (2.5YR 4/8); silty loam texture; weak and very fine, blocky sub-angular structure; slightly plastic and moderately sticky consistency when moist; few very fine pores; very fine, superficially distributed roots; no presence of macroorganisms; strongly acid pH (4.5); slightly effervescent reaction to decaying OM (H_2O_2); negative reaction to both free carbonates (HCl) and allophanes (NaF); abrupt and smooth boundary.
C1 98 – 128 cm	Dark red when moist (10R 3/6); silty loam texture; massive, structureless; moderately plastic and very sticky consistency when moist; few very fine pores; few very thin roots; no presence of macroorganisms; extremely acid pH (4.2); negative reaction to decaying organic matter (H_2O_2), free carbonates (HCl) and allophanes (NaF); abrupt and smooth boundary.
C2 128 – 138 cm	Yellowish red when moist (5YR 4/6); silty loam texture; massive, structureless; moderately plastic and very sticky consistency when moist; few very fine pores; very few and very fine roots; no presence of macroorganisms; extremely acid pH (4.2); negative reaction to both free carbonates (HCl) and allophanes (NaF).

Table 3. Soil description of *La Envidia* rubber plantation (Nechí).

Diagnostic horizons / Depth	Description
Ap 00-17 cm	Dark yellowish brown when moist (10YR 4/6); clay loam texture; fine and very fine granular structure; slightly plastic and moderately sticky consistency when moist; few very fine pores; many fine and very fine, superficially distributed roots; no presence of macroorganisms; extremely acid pH (4.0); very slightly effervescent reaction to decaying OM (H_2O_2); negative reaction to both free carbonates (HCl) and allophanes (NaF); gravel, rubble and quartz (1 to 5 cm Ø, 50%); gradual and smooth boundary.
bt 17-106 cm	Yellowish red when moist (5 YR 5/8), with 10% mottling: yellowish dun (10YR 5/8), olive yellow (2.5Y 6/8), yellow (2.5Y 7/8); sandy clay loam texture; weak and very fine granular structure; moderately plastic and very sticky consistency when moist; few very fine pores; very fine, superficially distributed roots; no presence of macroorganisms; extremely acid pH (4.0); negative reaction to decaying OM (H_2O_2), free carbonates (HCl) and allophanes (NaF); gravel, rubble and quartz (1 to 5 cm Ø, 40%); abrupt and smooth boundary.
C 106-132 cm	Dark brown when moist (7.5 YR 5/8), with <5% red mottling (2.5YR 5/8); silty loam texture; massive, structureless; very plastic and very sticky consistency when moist; few very fine pores; very few and very fine roots; no presence of macro-organisms; strongly acid pH (4.5); negative reaction to both free carbonates (HCl) and allophanes (NaF); gravel, rubble and quartz (1.5 to 3 cm Ø, <5%).

***Comment*:** Abundant presence of gravel, rubble and quartz was found from 33 to 101 cm deep, representing 50 to 60% of said layer. Quartz was observed to be highly weathered all along the profile, probably due to the removal of materials as part of anthropogenic (mining) activity. This might explain not only the presence of a deposit of gravel and rubble above the first layer of the profile, but also the large amounts of these materials contained in the first horizons and diminishing or disappearing towards the deepest layers, thus making them easier to penetrate.

Table 4. Soil description of *La Golondrina* rubber plantation (Caucasia).

Diagnostic horizons/Depth	Description
Ap 00-22 cm	Red color when moist (10R 4/6), with 10% reddish - black (10R 2.5/1) mottling; silty loam texture; moderate to strong, fine and very fine sub - angular blocky structure; slightly plastic and sticky consistency when moist; many fine and very fine pores; many fine and medium sized, superficially distributed roots; no presence of macro - organisms; extremely acid pH (4.0); very slightly effervescent reaction to decaying OM (H_2O_2) negative reaction to both free carbonates (HCl) and allophanes (NaF); 5-10 mm Ø gravel (30%); smooth and clear boundary.
bt 22-70 cm	Red color when moist (10R 4/8), with <5% reddish yellow (7.5 YR 6/8) mottling; silty clay loam texture; weak, very fine sub - angular blocky structure; moderately plastic and sticky consistency when moist; few very fine pores; very fine, superficially distributed roots; no presence of macroorganisms; extremely acid pH (4.0); negative reactions to decaying OM (H_2O_2), free carbonates (HCl) and allophanes (NaF); 3-5 mm Ø gravel (5%); gradual and smooth boundary.
C1 70-110 cm	Dark red color when wet (2.5YR 3/6); <5% dark brown (7.5 YR 5/6) mottling; silty clay texture; massive, structureless; very plastic and very sticky consistency when moist; very few fine pores; very fine, moderately abundant roots; no presence of macroorganisms; extremely acid pH (4.0); negative reactions to decaying OM (H_2O_2), free carbonates (HCl) and allophanes (NaF); 2 mm Ø gravel (10%); gradual and smooth boundary.
C2 110-120 cm	Red color when moist (10R 4/8), with <10% reddish yellow (7.5 YR 6/8) mottling; silty clay texture; structureless massive; very plastic and very sticky consistency when moist; few very fine pores; very few and very fine roots; no presence of macroorganisms; extremely acid pH (4.0); negative reactions to both free carbonates (HCl) and allophanes (NaF).

Comment: Mottling observed in the first layer may correspond to ashes resulting from burnt trees.

Soil physical properties

Soil moisture underwent slight modifications along the vertical soil profiles (Table 5). The lowest moisture content record came from La Envidia, at the 0 - 30.5 cm deep layer; while the highest one corresponded to La Golondrina (at the 61.0 - 91.5 cm deep layer). These observations can be confirmed by comparing hydraulic conductivity (Table 6) to soil moisture retention data (Figure 2). These results are well explained by texture and soil structure, although water retention can also be influenced by other biotic and abiotic factors (Hernández and Sánchez, 2012). In this regard, the silty clay loam observed in La Envidia, together with the presence of gravel, rubble and quartz from 17 to 106 cm deep (Table 2) and its low OM content (Table 7), all adversely affect the water retention capacity of the soil (Murray-Núñez *et al.,* 2011). In turn, this situation favors the possibility that the rubber plantations established there face stressful events in times of prolonged drought.

Mechanical resistance to penetration did not exceed 2.0 MPa in any case (Table 5), which is the critical threshold above which radical growth is affected (Martino and Shaykevich, 1994). Hence, this constitutes a desirable soil attribute for the proper development of plants. In this regard, the *BCA* region soils are relatively advantageous for rubber plantations when compared to those in other areas of the country where the trees support the weight of grazing cattle (Rosas *et al.,* 2016). Regarding bulk density, the values shown in Table 6 match those mentioned by Alvarado and Forsythe (2005) for Ultisols and are consistent with expectations for fine textured soils. The differences observed in this parameter across the studied landscapes are inversely correlated to OM contents (Table 7). In this sense, low OM content observed at La Envidia contrasts with the elevated value obtained at La Golondrina, a circumstance that leads to increased biological activity in the root zone, which, in turn, favors the development of roots (Chinchilla *et al.*, 2011). For its part, porosity varied between 64.7% (Villa Gina), 63.4% (La Golondrina) and 49.3% (La Envidia). This disparity is attributed to the higher OM content and lower bulk density values of Villa Gina and La Golondrina, as compared to those recorded at La Envidia, involving dissimilarities in form, grade and size of the aggregates (Cuevas *et al.*, 2006). Another observation related to porosity and, therefore, to OM content, is the advantage that these attributes confer to Villa Gina and La Golondrina. In effect, water retention in the soil is a function of the specific surface of each soil and, consequently, of pore amount, size and distribution (Krull *et al*., 2004).

Table 5. Soil moisture content in three rubber plantations of the Bajo Cauca Antioqueño region (Colombia).

Farm	Soil moisture		Resistance to penetration	
	Depth (cm)	(%)	Depth (cm)	(MPa)
Villa Gina (Tarazá)	0.0 – 30.5	32.1	0.0 – 10.2	0.7
	30.5 – 61.0	40.6	10.2 – 20.3	1.1
	61.0 – 91.5	42.2	20.3 – 30.5	1.6
La Envidia (Nechí)	0.0 – 30.5	21.5	0.0 – 10.2	1.1
	30.5 – 61.0	25.7	10.2 – 20.3	---
	61.0 – 91.5	25.2	20.3 – 30.5	---
La Golondrina (Caucasia)	0.0 – 30.5	46.2	0.0 – 10.2	1.1
	30.5 – 61.0	47.1	10.2 – 20.3	1.2
	61.0 – 91.5	54.5	20.3 – 30.5	1.4

Table 6. Soil physical properties in three rubber plantations of the Bajo Cauca Antioqueño region (Colombia).

Farm	Hydraulic conductivity (cm min^{-1})	Bulk density (mg m^{-3})	Total porosity (%)
Villa Gina (Tarazá)	0.05	1.01	64.7
La Envidia (Nechí)	> 5.0	1.36	49.3
La Golondrina (Caucasia)	0.03	0.98	63.4

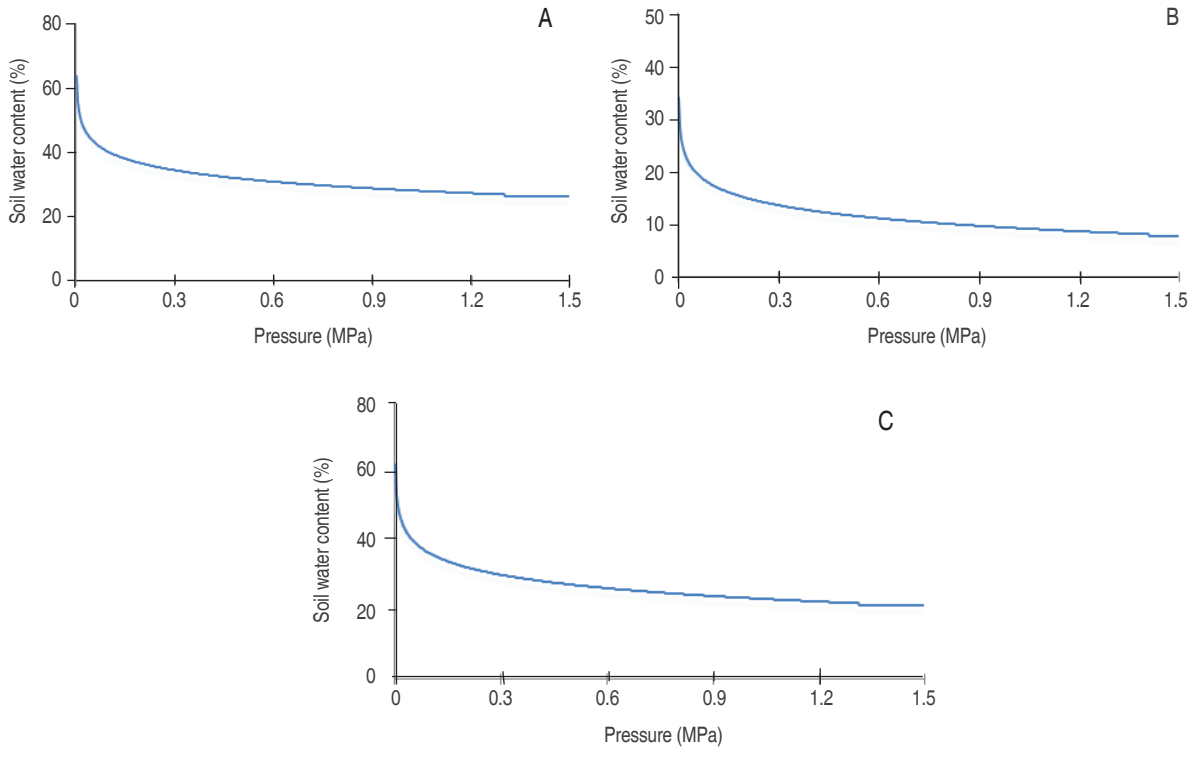

Figure 2. Soil moisture retention curves from three rubber plantations in the Bajo Cauca Antioqueño region (Colombia). A. *Villa Gina* (Tarazá); B. *La Envidia* (Nechí); C. *La Golondrina* (Caucasia).

Soil fertility

Table 7 shows the nutrient content data of the soils under study. Together with the udic moisture regime of the region (Malagón, 1998), these results confirm the great limitations of Ultisols, which include strong acidity (Table 2), Al toxicity, low ECEC and low fertility. OM content was found to be low in Villa Gina and La Envidia, and medium in La Golondrina, where this condition favors the physical, chemical and biological properties of the soil (Julca-Otiniano *et al*., 2006), in turn facilitating better nutrient availability for rubber plantations. Moreover, the low content of organic C makes *BCA* soils more susceptible to erosion, due to the low degree of stability, have a minimum capacity to retain moisture, minor gas mobility and reduced biological activity (Martínez *et al*., 2008).

The limited presence of nitrifying bacteria in Ultisols results in poor nitrification, which, in turn, determines low N availability for rubber cultivation in the landscapes under study (Salinas and Valencia, 1983; Montaño and Sánchez-Yáñez, 2014). For the three rubber plantations, the laboratory analysis showed very low P levels (2 mg kg^{-1}), which are explained by the high degree of weathering of Ultisols and the strong affinity of this element with Fe and Al, with which it forms insoluble precipitates (Chinchilla *et al.,* 2011). In this case the use of rock phosphate may be effective, particularly if mycorrhizal fungi are inoculated because rubber plant growth and nutrient uptake is promoted by them (Sosa *et al*., 2009).

While La Golondrina showed the highest K contents, the other two sites registered critical levels that are probably affecting plant growth and crop yield by the rubber trees planted there (Pervez *et al*., 2004). The contents of Ca in the three studied landscapes were found to be low (Villa Gina) to very low (La Envidia and La Golondrina) (Minagricultura, 2014). In acid and intensely weathered soils the deficiency of this mineral, together with low P availability and Al toxicity, certainly limits fertility, since it constitutes a chemical barrier that prevents root growth and reduces both microorganism populations and their activity (Salinas and Valencia,

1983). Mg was found to be at very low levels in the three studied rubber plantations, which is related to the high degree of weathering and the washing of bases observed in the region (Paniagua-Vásquez and Toruno-Gutiérrez, 2004). The concentration of S was medium at Villa Gina and La Envidia, while its deficiency in La Golondrina may be due to the high solubility exhibited there by this nutrient (Salinas and Sanz, 1981).

Table 7. Soil chemical characteristics in three rubber plantations of the Colombian Bajo Cauca Antioqueño region.

Element	Villa Gina (Tarazá)	La Envidia (Nechí)	La Golondrina (Caucasia)
OM (%)	2.8	1.6	3.3
OC (%)	1.6	0.9	1.9
$N\text{-}NO_3$ (mg kg^{-1})	8	1	6
$N\text{-}NH_4$ (mg kg^{-1})	30	19	30
P (mg kg^{-1})	2	2	2
K ($cmol_c$ kg^{-1})	0.10	0.03	0.33
Ca ($cmol_c$ kg^{-1})	3.3	0.1	0.79
Mg ($cmol_c$ kg^{-1})	1.5	0.04	0.88
S (mg kg^{-1})	19	16	3
Mn (mg kg^{-1})	28	1	21
Zn (mg kg^{-1})	2	1	1
Cu (mg kg^{-1})	5	1	2
B (mg kg^{-1})	0.17	0.17	0.33
Fe (mg kg^{-1})	14	213	124
Al ($cmol_c$ kg^{-1})	0.2	1.6	6.5
ECEC ($cmol_c$ kg^{-1})	5.1	1.8	8.5

Regarding micronutrients, a marked deficiency of Mn was observed in the soils of *La Envidia*, probably resulting from the acidity of the substrate, which tends to dissolve this metal (Matini *et al*., 2011) and this has been leached out from the soil. Regarding Zn, its outstanding deficiency in the soils of *La Envidia* and *La Golondrina* is attributable to the sandy nature of the soil, acidity and low OM content (Oku *et al*., 2012). In contrast, the concentration of Cu was found to be at acceptable levels in *Villa Gina* and *La Golondrina*, perhaps because the slightly higher levels of organic C observed there increased the content of this nutrient (Mohd-Aizat *et al*., 2014). The soil available B content was found to be very low in the three rubber plantations, probably because this element is adsorbed onto the surfaces of Fe and Al oxides, which reduces its availability (Salinas y Valencia, 1983; Calbaceta and Molina, 2006). It is worthwhile noting the elevated levels of Fe and Al. The former, which is responsible for the red color that features the studied soils, is released from their primary minerals and then progressively oxidized (Malagón *et al*., 1995). In turn, Al results from advanced soil weathering, which releases Al ions from the network formed by clay silicates (Casierra-Posada and Aguilar-Avendaño, 2007). These ions when present in the soil interfere the transport and usage of essential nutrients (P, K, Ca, and Mg) and inhibit microbial processes that supply nutrients to the plant (Campillo and Sadzawka, 2008).

Finally, the low ECEC observed in the present study correlates with clay mineral composition and very low organic C content. In Ultisols, the clay fraction is mainly composed of kaolinite or oxyhydroxides of Fe and Al, while the sand and silt fractions are dominated by quartz and minor amounts of mica, feldspar, ferromagnesium and other weathering resistant minerals. This results in soils with limited ECEC and restricted supply of bases, both resulting from mineral weathering (Prasetyo *et al*., 2001; Durango, 2014).

CONCLUSIONS

The rubber planted soils of the Bajo Cauca Antioqueño region are deep and well drained. Through typological analysis, they can be said to belong to the order Ultisols. Iron accumulation gives them a red and yellowish hue. They are very acid and possess low natural fertility. Their texture was generally found to be silty loam and clay loam. Their content of OM and exchangeable bases is low, as well as the availability of P and K. The levels of Fe and Al are very high. Their mineralogical composition and low content of organic C contribute to low ECEC values. Notwithstanding, the studied area is potentially useful for the development of perennial plantations such as rubber. An adequate agronomic management of these soils should be aimed at obtaining sustainable productivity. As such, it must be capable of developing and maintaining fertility through liming, OM incorporation and appropriate application of the correct amounts of phosphate and potassium fertilizers as well as biofertilizers based on arbuscular mycorrhizal fungi.

ACKNOWLEDGEMENTS

The authors would like to thank the Fund for Science, Technology and Innovation of the General System of Royalties of the department of Antioquia (*Fondo de Ciencia, Tecnología e Innovación del Sistema General de Regalías del Departamento de Antioquia*) (2013 budget assignment), for the financial support of the project "Evaluación y Diagnóstico de los Limitantes Nutricionales en la Producción de Caucho (*Hevea brasiliensis* Muell Arg.) en el Bajo Cauca Antioqueño" (Hermes UNAL code 17150). The project is part of special cooperation agreement 46000001081, subscribed by Universidad Nacional de Colombia – Medellín *campus*, Universidad de Antioquia, Universidad EAFIT, SENA, CORPOICA, ASCABIA, ASCULTICAUCHO and the Departmental Governor's Office of Antioquia.

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Revista Facultad Nacional de Agronomía

Biological, botanical and chemical alternatives for the control of blackberry *(Rubus glaucus* Benth.*)* diseases

UNIVERSIDAD NACIONAL DE COLOMBIA

Alternativas biológicas, botánicas y quimicas para el control de enfermedades en el cultivo de la mora (*Rubus glaucus* Benth)

doi: 10.15446/rfna.v70n2.64521

Oscar Darío Hincapié Echeverri[1]*, Alegría Saldarriaga Cardona[2], Cipriano Díaz Diez[2]

ABSTRACT

Keywords:
Fruit trees
Phytopathology
Antagonistic fungi
Biocontrollers
Integrated management
Organic management

In order to control the main diseases that affect blackberries (*Rubus glaucus* Benth.), a research in which 12 treatments to San Antonio ecotype plants originated *in vitro* was conducted. These treatments were: 1: *Trichoderma harzianum* + *Trichoderma koningii* (Tropical Fungus), 2: *Trichoderma* sp. (Bioprotection), 3: *Trichoderma koningiopsis* (Th003 Trichoderma), 4: *Trichoderma asperellum* (Th034 Trichoderma), 5: *Trichoderma asperellum* (T-30 Trichoderma), 6: *Trichoderma asperellum* (T-98 Trichoderma), 7) *Burkholderia cepacia* (Botrycid), 8: Extract of *Swinglea glutinosa* (Ecoswin), 9: Traditional farming treatments (Mancozeb, Propamocarb), 10: Chemical products applications (Mancozeb, Mandipropamida, Carbendazim, Propamocar and Metalaxil+Mancozeb) alternated according to the impact of the disease, 11: Chemical products applications alternated with organic products according to the suppliers recommendations and presence of the diseases, 12: Absolute control, no treatment was applied to the plants. The applications were carried out every 15 days, each plant was an experimental unit and each treatment was made of five experimental units. 12 treatments were made through a RCBD (randomized complete block design) with three repetitions for a total of 15 experimental units per treatment. The assessments were performed every 8 days, and the variables were: number of healthy and sick fruits/treatment, costs/treatment and gross profit. A variance analysis and a Tukey test 5% were made. The best treatments were T11 (rotation of chemicals with biological products), T10 (rotation of chemical products according to the impact of the disease) and T3 (*T. koningiopsis*); considering the obtained performance/treatment, treatment cost and profit.

RESUMEN

Palabras claves:
Frutales
Fitopatología
Hongos antagónicos
Biocontroladores
Manejo integrado
Manejo orgánico

Para el manejo de las principales enfermedades de la mora (*Rubus glaucus* Benth.), se realizó una investigación donde se aplicaron 12 tratamientos a plantas ecotipo San Antonio, procedentes de cultivo *in vitro*. Los tratamientos fueron 1: *Trichoderma harzianum+Trichoderma koningii* (Hongos del Trópico), 2: *Trichoderma sp.* (Bioprotección), 3: *Trichoderma koningiopsis* (Th003 Trichoderma), 4: *Trichoderma asperellum* (Th034 Trichoderma), 5: *Trichoderma asperellum* (T-30 Trichoderma), 6: *Trichoderma asperellum* (T-98 Trichoderma), 7: *Burkholderia cepacia* (Botrycid), 8: Extracto de *Swinglea glutinosa* (Ecoswin), 9: Tratamiento tradicional del agricultor (Mancozeb, Propamocarb), 10: aplicaciones de productos químicos (Mancozeb, Mandipropamida, Carbendazim, Propamocar y Metalaxil+Mancozeb), en rotación según incidencia de las enfermedades, 11: Aplicación de productos químicos en rotación con biológicos, según recomendaciones de los proveedores y presencia de las enfermedades, 12: Testigo absoluto, no se aplicó ningún tratamiento a las plantas. Las aplicaciones se realizaron cada 15 días, cada planta fue una unidad experimental y cada tratamiento estuvo compuesto por cinco unidades experimentales, se realizaron 12 tratamientos en un diseño de BCA con tres repeticiones, para un total de 15 unidades experimentales por tratamiento. Las evaluaciones se realizaron cada ocho días y las variables consideradas fueron: número de frutos sanos y enfermos/tratamiento, costos/tratamiento y ganancia bruta. Se realizó un análisis de varianza y una prueba de Tukey 5%. Los mejores tratamientos fueron el T11 (rotación de productos químicos con biológicos), el tratamiento T10 (rotación de productos químicos según la incidencia de las enfermedades) y el tratamiento T3 (*T. koningiopsis*); considerando los rendimientos obtenidos/tratamiento, el costo del tratamiento y la ganancia alcanzada.

[1] Corporación Colombiana de Investigación Agropecuaria - Corpoica. C.I. Tibaitatá. A.A. 240142 Las Palmas, Bogotá, Colombia.
[2] Corporación Colombiana de Investigación Agropecuaria - Corpoica. C.I. La Selva. A.A. 100. Rionegro, Colombia
* Corresponding author <ohincapie@corpoica.org.co>

Received: May 31, 2016; Accepted: December 21, 2016
Rev.Fac.Nac.Agron. 70(2): 8169-8176. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

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Mora de Castilla (*Rubus glaucus* Benth.) is affected by diverse diseases mostly caused by fungi, amongst these diseases there is the Anthracnose, which is caused by *Glomerella cingulate* (Stoneman) Spauld. and Schrenk. (anamorph *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.), *Colletotrichum acutatum* Simmonds., *Colletotrichum boninense* Moriw., Sato and Tsukib.; these pathogens affect stems, petioles and reproductive structures causing lesions that can end in the death of the plant's branches affecting the crop production (Saldarriaga *et al.,* 2008; Saldarriaga, 2006; Afanador *et al*., 2009; Tamayo, 2009; Rueda, 2010). The Fungus that causes the Anthracnose can also damage flower buds, tender shoots, stalks and fruits (Franco and Giraldo, 2000). When the fungus infects the plant's side branches, this creates a deformation on the group of flowers, the tender shoots blacken, and the branches die in a descendent and progressive way (Castro and Díaz, 2001). In the inflorescences, this infection appears as a necrosis of the tissues in which the fungus sporulates (Rueda, 2010). In ripen fruits, the disease appears sporadically. In this case, the affected fruits show dehydration, necrosis and wet rot in the presence of some fungal structure (Saldarriaga and Bernal, 2000; Botero *et al*., 2002). This disease deteriorates between 50 and 70% of the cultivated blackberry plants' stems and causes a loss of a 5% of the pathogens-affected fruits (Tamayo, 2009). The incidence of this disease has been registered in a 52% under the conditions given by the blackberry crops in the Colombian coffee belt region (Botero *et al*., 2002).

The gray mold caused by *Botrytis cinerea* Pers, Ex. Fr., is another important disease that appears during the stage of production and postharvest of the blackberry. This fungus affects the flower buds since their opening and appears in the fruition and ripening, causing necrosis and mummification of the fruits (Forero de La-Rotta, 2007) (Quinatoa, 2015). In the presence of humidity, the affected fruits show a growth in the fungus with a velvety aspect of colors gray, brown or olive green. The fruits dry and mummify stuck to the cluster (Saldarriaga and Bernal, 2000; Tamayo, 2009). The fungus can also affect leaves, flowers and stalks (Franco and Giraldo, 2000). This disease has created losses between 50 to 76% of the harvested fruit affected by pathogens (Tamayo and Peláez, 2000).

The downy mildew caused by *Peronospora* Corda affects the stems, stalks, flower buds, flowers and fruits. The sick stems and stalks show purple discolorations with whitish lesions on which a downiness of light gray color grows (Saldarriaga and Bernal, 2000; Tamayo, 2003). The flowers show yellowing then their petals get dry to fall later on. The sepals show a light brown to black color lesion which advances until the flower buds mummification (Tamayo, 2009). In fruits, the fungus causes an irregular development, uneven ripening, and loss of fruit brightness, which depreciates the fruit in its commercialization. The green fruits stop growing (Saldarriaga and Bernal, 2000; Franco and Giraldo, 2000). This disease can cause losses of a 20 to 30% of the harvested fruits (Tamayo and Peláez, 2000).

Another disease that can affect the blackberry is the powdery mildew. This is caused by the fungus *Oidium* Link. This infection appears mainly in young leaves through deformation (leaf curling), it is associated to the presence of chlorotic, irregular and diffused areas that can be seen on the surface of the foliar laminae. Occasionally, the leaves are covered on a fine white dust that corresponds to the growth and sporulation of the fungus. The yellow rust is also an important disease; this one is caused by the fungus *Gerwasia lagerheimii* (Magnus) Buritica, it appears in the form of pustules of orange color in the stems, stalks, the reverse of leaves and in the fruits (Franco and Giraldo, 2000; Tamayo, 2009).

There are reports of foliar conditions caused by the fungi *Septoria* Sacc, *Phyllosticta* Pers, *Alternaria* Nees, which importance is still secondary due to the low incidence of the diseases caused by them (Tamayo, 2009). The stems and roots of the blackberry also present some diseases caused by fungi such as: *Coniothyrium fuckelii* Sacc, *Verticillium albo-atrum* Reinke and Berth, *Rosellinia* De Not, *Fusarium roseum* Link: Fr. and *Fusarium oxysporum* Schlechtend. Fr, which importance has not been established yet (Tamayo, 2009).

Traditionally the treatment of the diseases of the *Mora de Castilla* has been based on the development of some cultural practices and the periodic application of different fungicides —there is no official record of most of them being used for the blackberry—. The current tendencies in food production are oriented towards getting products with less pesticide charges, besides

the demands on environmental conservation and food harmlessness. Nowadays, in the field of blackberry harvesting, the use of fungicides is starting to be restricted or forbidden, such as the products based on Clorotalonil, Mancozeb, Propineb, Metalaxil + Mancozeb, Propineb + Cymoxanil (Arroyave and Salazar, sf., quoted by Díaz, 2009). Research institutions and some companies in the commercial sector are developing products of botanical origins such as vegetables extracts, and biological origins such as antagonistic microorganisms with the potential use of the control of the diseases caused by fungi of the genus *Colletotrichum*, *Botrytis*, *Peronospora*, *Oidium* (Restrepo *et al*., 2001; Agrobiológicos Safer and Natural Control Sf, Vélez and Estrada sf, Bioprotección sf, Ecoflora, 2007; Velosa *et al.,* 2002; 2003, Castro and Díaz, 2001).

This situation has created the need for more research on new products and management strategies that allow the offering to the blackberry producers of more efficient and sustainable alternatives to the integral management of the main diseases that affect this crop, contributing to the obtaining of a product harmless to the consumers. With the results from this research, some key knowledge is provided to the creation of blackberry disease control proposals, considering among others, the efficiency of the products and the economic sustainability to satisfy the demands of the food and agriculture chain of the blackberry.

MATERIALS AND METHODS

The in-field observations were carried out in a period of 20 months in Corpoica R.C. La Selva, Rionegro, Antioquia, under conditions of a Lower Montane moist forest (LM-mf). 12 treatments were applied to blackberry plants of the San Antonio ecotype, originated *in vitro*. The evaluated treatments were the following: T1: *Trichoderma harzianum*+*Trichoderma koningii* (commercial product of *Hongos del Trópico*), T2: *Trichoderma* sp. (comercial product of *Bioprotección*), T3: *Trichoderma koningiopsis* (Th003 Trichoderma, product of CORPOICA), T4: *Trichoderma asperellum* (Th034 Trichoderma, product of CORPOICA), T5: *Trichoderma asperellum* (T-30 Trichoderma, product of the Corporación para Investigaciones Biológicas – CIB, in scaling process), T6: *Trichoderma asperellum* (T-98 Trichoderma, product of the CIB, in scaling process), T7: *Burkholderia cepacia* (Botrycid, commercial product of *Safer*), T8: extract of *Swinglea glutinosa* (EcoSwing® commercial product of *Ecoflora*), T9: corresponds to the traditional treatments of the farmer based on the applications of chemical products with active ingredients such as (Mancozeb) and (Propamocarb), T10: Application of chemical products (the products with the following active ingredients were applied: (Mancozeb), (Mandipropamida), (Carbendazim), (Propamocarb), (Metalaxil+Mancozeb) alternating according the effect and incidence of the blackberry diseases, T11: Application of chemical products alternating with biological products (bio-control microorganisms and vegetal extracts) according to the recommendations of the suppliers and the phenological state of the plants; the following products were applied: extract of *Swinglea glutinosa*, *Burkholderia cepacia*, *Trichoderma koningiopsis* (Th003), *Trichoderma asperellum* (T-98), *Trichoderma* sp., Propamocarb, Metalaxil +Mancozeb, Mandipropamida, taking into account the presence and incidence of the diseases, T12: Absolute control, no treatment was applied to the plants. The 12 treatments were distributed in three completely randomized blocks, each plant was an experimental unit and each treatment was made of five experimental units.

The plants that underwent the treatment were surrounded by blackberry plant sources of inoculum, for a total of 270 plants sown with a distance of 2 m x 2 m between plants and 3 m x 3 m between furrows, in an area of 1530 m^2 (Díaz, 2009; Hincapié, 2010). Previous to the crops' establishment, a chemical analysis of the soil was made which was used as a base to the fertilization process. The cultural labors proper to the crops were made such as the *plateo* (keeping the young plants clear of weeds), weed control, pruning, wiring on a modified double T trellis system, maintenance pruning (leaving 5 stems per plant), sanitary pruning, and destruction of the pruning remainders. The plot was monitored on a weekly basis and the application of the treatments started once a 50% of the plants were blossomed. These applications were carried out every 15 days, using the doses recommended by the products manufacturers and traders. The evaluations were carried out every eight days, by collecting and separating the healthy fruits from the sick on each type of treatment in order to be quantified in the laboratory later on through the processes of counting, weighing, classification and registry of the fruits affected by the diseases. The following were taken into consideration: the amount of healthy fruits

and their weight, the number of fruits affected by the gray mold (*Botrytis cinerea*), downy mildew or peronospora (*Peronospora* sp.), and the anthracnose (*Colletotrichum* sp.). For each of the treatments the performance per treatment (kg ha^{-1}) was estimated. For the mentioned variables, an analysis of variance and the medians separation according to the Tukey test on a 5% level were conducted. Besides, the cost of the different treatments was calculated and the gross profit was estimated per hectare sown (1666 plants). The experiment was suspended once the plants reached the 11 month of the productive stage (the peak or production under the agro-ecological conditions where the plot was established is reached 18 months after the beginning of the productive stage) (Díaz, 2009; Hincapié, 2010).

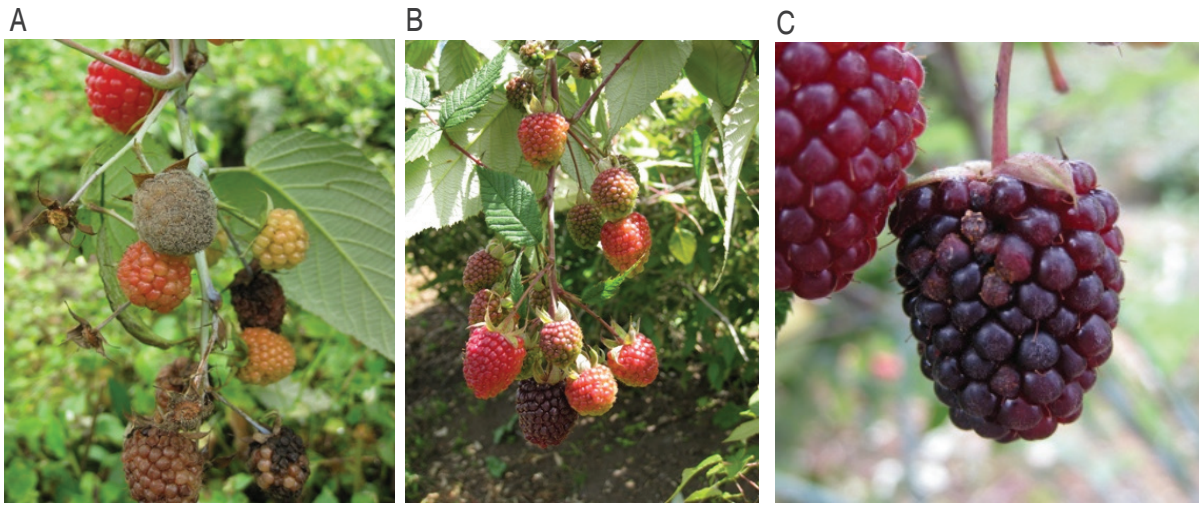

Figure 1. A. Blackberry fruits affected by gray mold (*Botrytis cinerea*). B. Blackberry fruits affected by downy mildew (*Peronospora* sp). C. Blackberry fruits affected by Anthracnose (*Colletotrichum* sp).

RESULTS AND DISCUSSION

The results obtained through this research are the following: on the Absolute Control treatment (T12), the treatments that showed a higher percentage of healthy fruits, with no significant statistical differences, by the gray mold, downy mildew and Anthracnose diseases (Figure 1) were: T11, T10, T3 and T9 (Table 1). For this group of treatments, the percentage of sick fruits was between 35 and 39%; whereas the Absolute Control group presented an 81% of sick fruits. In this same group of treatments, the best results for the gray mold management were achieved with the use of T3 and T11 (with percentages between the 2.2 and 2.5%) in contrast to the Control group that presented a 15%. For the downy mildew the percentage of sick fruits was between the 32.3 and 36%, being the best treatments the T10, T11 and T9 in contrast to the Control group which presented a 64.2% (Table 1). Regarding the Anthracnose, the percentage of affected fruits was less than 2.1%. Treatment T8 did not show any difference from the Absolute Control T12, but both presented differences from the rest of treatments (Table 1).

In addition to this, after analyzing all treatments, and independently the behavior of the diseases, the following was observed: for the gray mold the best treatments were the T1, T3 and T11 which presented the lowest percentage of fruits affected by the disease (2.2% to 2.5%) (Table 1); to this group the treatments that followed are T7, T5, T10, T4 and T8 with percentages between the 3 and 4% of the fruits affected by the disease with no significant differences between them. Treatments T6, T9 and T12 were the less efficient. Treatment T12 showed significant differences from all treatments and reached the highest percentage of fruits affected by *Botrytis cinerea*, corresponding to a 15%. Figure 1A illustrated the symptoms of the fruit affected by gray mold, these are consistent to a soft rot in which appears a growth of grayish color that corresponds to the sporulation of the fungus.

Table 1. Behavior of the different treatments according to the percentage of healthy and sick fruits.

Treatments	Healthy fruits (%)	Sick fruits (%)	Fruits with gray mold (%)	Fruits with downy mildew (%)	Fruits with anthracnose (%)
T1	48.69 b	51.31 b	2.27 d	46.19 bc	0.71 bc
T2	46.70 b	53.09 b	5.64 b	46.44 bc	1.05 bc
T3	61.02 a	39.00 c	2.28 d	36.00 c	0.71 bc
T4	49.90 b	50.10 b	4.18 bc	44.73 bc	1.19 b
T5	42.26 b	53.74 b	3.84 bc	49.05 b	0.85 bc
T6	41.92 b	58.08 b	4.81 b	52.14 b	1.16 b
T7	42.68 b	57.36 b	3.74 bc	52.76 b	0.95 bc
T8	44.86 b	55.14 b	4.24 bc	48.80 b	2.10 a
T9	60.62 a	39.38 c	5.00 b	33.89 d	0.58 bc
T10	63.07 a	36.93 c	3.95 bc	32.35 d	0.64 bc
T11	64.34 a	35.66 c	2.56 cd	32.37 d	0.74 bc
T12	19.00 c	81.00 a	15.04 a	64.24 a	1.72 a
Variance coefficient	11.47%	11.06%	20.69%	11.33%	44.78%

Results with equal letters have no significant difference between treatments.

Regarding the downy mildew, this was the disease that showed the high percentages of affected fruits (32% to 64%). With treatments T10, T11, T9, the lowest percentages of sick fruits were obtained, around 32% (Table 1). For the other treatments the percentage of sick fruits was between the 36 and 52.7%. Some significant differences were established with the Absolute Control group, which reach a 64% of fruits with symptoms of the disease. This disease manifests with bad filled drupes, uneven ripening, irregular development (Figure 1B), and the fruits harden when touched.

Regarding the Anthracnose, this disease showed the lower percentage of affected fruits (less than 2.1%) Other studies have reported losses by Anthracnose estimated in approximately a 5% of the fruits affected by different diseases (Tamayo, 2009). In the fruits the disease appears sporadically and the symptoms correspond to drupes dehydration, necrosis (Figure 1C), and wet rot with presence of fungal structures (Saldarriaga and Bernal, 2000).

During this study, besides the behavior of the diseases in front of the different treatments, the production (kg of healthy fruits per treatment ha^{-1}), the production costs (assuming $2000 COP as the sale value of a kilo of blackberry), and the cost of the treatment were considered. Table 2 registers the respective values and costs for each of the treatments.

The highest production (t ha^{-1} per year) was reached on treatments T10, T9 and T11; however, when the costs analysis was made, it showed that the value of consumables for some treatments was considerable and affected negatively the profit; leading in some moments to produce losses, like can be seen on treatment T9.

T11 (chemical products alternating with biological products and vegetal extract) was considered as a promising

Table 2. Costs and gross profit for every treatment.

Treatment	Performance (t ha^{-1} $year^{-1}$)	Production value ($cop)	Cost of treatment ($cop)	Gross profit
T1	2.50	4,992,737	12,856,168	-7,863,431
T2	2.31	4,626,915	1,455,272	3,171,643
T3	2.68	5,365,805	121,285	5,244,520
T4	2.43	4,858,596	121,285	4,737,311
T5	2.30	4,595,323	621,973	3,973,350
T6	2.21	4,413,292	621,973	3,791,319
T7	1.91	3,819,759	5,146,826	-1,327,067
T8	1.85	3,691,563	8,816,472	-5,124,909
T9	7.24	14,482,753	15,001,997	-519,244
T10	8.25	16,507,466	8,915,720	7,591,746
T11	5.54	11,076,267	5,206,295	5,869,972
T12	1.26	2,517,418	0	2,517,418

treatment due to the gross profit and the possibility of reducing the charge of chemical products and the addition of biological products, which can contribute to the environmental conservation and the obtaining of a harmless product. In order to apply this treatment it's important to monitor the diseases regarding their happening and severity to define the type of product that will be used, besides the weather conditions at the moment of application have to be considered. Treatment T10 (chemical products rotation according to the happening of diseases) allowed to obtain the highest production and gross profit (Table 2), and a most rational management of the diseases but it needs the implementation of a monitoring component of the diseases as a selection criteria of the products to apply. Treatment T3 (Th003 - *T. koningiopsis*) can be an alternative to contribute to the integrated management especially in crops with a high incidence of gray mold. Currently, the laboratory of biological control of Corpoica has a wet powder (WP) product made from *Trichoderma koningiopsis* Th003 named "Tricotec". This bioinsecticide has demonstrated excellent results in vegetable crops for the pathogens control such as *Rhizoctonia solani*, getting a reduction of the disease of a 71% in Chonto tomato (Gómez *et al*., 2011; Gómez, 2012). It has also shown a high efficacy in the control of *Botrytis cinerea* and *Oidium lycopersicum* in tomato by being applied to the phyllosphere; nevertheless, studies on the valuation of technology indicated a high economic feasibility for the soil application and the foliar application (Gómez *et al*., 2011). According to Gómez (2012), in the trials made on lettuce with the bioinsecticide (WP) *T. koningiopsis* Th003 syptoms of the pathogen *Botrytis* spp. did not appear.

CONCLUSIONS

The best results were obtained through the following treatments: T11 (use of chemical products alternating with biological products —biocontrollers and botanic extracts—) followed by treatments T10 (chemical rotation of products according to the happening of diseases), and the treatment T3 made of *Trichoderma koningiopsis* (Th003); all of this considering the obtained performance/ treatment, the cost of each treatment and the gross profit among others.

The treatments evaluated under experimental conditions were inefficient for the control of the downy mildew (*Peronospora* sp.), getting better results on the control of the gray mold and the Anthracnose of the fruit when said treatments are applied combining the opportune and adequate implementation of the cultural labors (sanitary and ventilation pruning, harvest and destruction of sick fruits, weed control, fertilization and management of pruning residues).

The products' evaluation on different grades of scaling does not allow to make the best comparison due to the possibility of having given more advantages to certain products in relation to other products that were on inferior stages of manufacture.

The low performance of the crops are explained considering that experimentally the work was carried out with only five stems per plant, and besides, the crops corresponded to the beginning of the plants' productive stage and did not reach the peak of the harvest.

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Research article

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Revista Facultad Nacional de Agronomía

Growing of coffee seedlings on different substrates and fertilized with lithothamium

UNIVERSIDAD NACIONAL DE COLOMBIA

Producción de plántulas café cultivado en diferentes tipos de substratos y fertilizado con lithothamnium

doi: 10.15446/rfna.v70n2.64522

Walter Danilo Maradiaga Rodriguez[1]*, Adão Wagner Pego Evangelista[2], Jose Alves Junior[2] and Marcus Vinicius Honorato[2]

ABSTRACT

The coffee seedling production quality is essential to ensure crop development in field. Thus, the objective of this study was to evaluate the effect of two substrates (cattle manure and filter cake) interacting with four levels of organic fertilizer with lithothanmium in coffee seedlings var. Lempira production. A completely randomized experimental design was used with factorial arrangement of treatments 5 x 2, five levels of lithothamnium (0, 1.75, 3.50, 5.25, and 7 kg m^{-3} of substrate), two substrates (cattle manure and filter cake) and three replicates. The variables evaluated were: plant height, number of leaves, stem diameter, and root length. The treatments produced significant effects on the initial development parameters of the coffee seedlings. The best results in plants grown were obtained in cattle manure substrate with 5.25 kg m^{-3} of lithothamnium, and decreased with levels higher than 1.75 kg m^{-3} of lithothanmium in filter cake substrate.

Keywords:
Coffea arabica L.
Filter cake
Cattle manure
Nursery
Fertilizing

RESUMEN

La producción de plantas vigorosas constituye un requisito indispensable para garantizar el desarrollo de los cultivos en el campo definitivo. Así, el presente trabajo tuvo como objetivo evaluar el efecto de dos sustratos (estiércol de corral y torta de filtro) y cuatro dosis de fertilizante orgánico con lithothanmium en la producción de plántulas de café var. Lempira. Se utilizó un diseño experimental completamente al azar con arreglo factorial de los tratamientos 5 x 2: 5 doses de lithothanmium (0, 1,75, 3,50, 5,25 y 7 kg m^{-3} de sustrato), dos sustratos (estiércol de corral y torta de filtro) y tres repeticiones. Las variables evaluadas fueron: altura de la planta, número de hojas, diámetro de tallo y crecimiento de raíz. Los tratamientos produjeron efectos significativos en los parámetros de desarrollo inicial de las plántulas de café. Los mejores resultados en plantas cultivadas se obtuvieron en el sustrato de estiércol con 5,25 kg m^{-3} de lithothamnium y disminuyeron con dosis superiores a 1,75 kg m^{-3} de lithothamnium en sustrato de torta de filtro.

Palabras claves:
Coffea arabica L.,
Torta de filtro
Estiércol de corral
Vivero
Fertilización

[1] Escola de Agronomia. Universidade Federal de Goiás. Caixa Postal 131. CEP 74690-900. Goiânia, GO, Brasil.
[2] Núcleo de Pesquisas e Recursos Hídricos do Cerrado. Escola de Agronomia. Universidade Federal de Goiás. Caixa Postal 131. CEP 74690-900. Goiânia, GO, Brasil.
* Corresponding author: <wmaradiaga@unag.edu.hn>

Received: March 4, 2016; Accepted: January 10, 2017
Rev.Fac.Nac.Agron. 70(1): 8177-8182. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

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In recent years, there has been a significant expansion of the market for organic products (Fibl and Ifoam, 2015). The socio cultural development of modern societies has led to the emergence of new consumption patterns in the food market (Rezende *et al*., 2010). The awareness of the need to preserve the environment and the growing demands for healthy and quality food have potentiated the organic-ecological production. The high cost of conventional agriculture has led several farmers to rethink their way of producing up to the use of innumerable sources of organic waste as alternatives to fertilization (Rezende *et al*., 2010).

The need for the coffee sector, to increase the productive efficiency accompanied by reduction of costs of production, for greater competitiveness, causes that the necessity arises to look for new alternatives of production.

It is known that the production of coffee quality seedlings is an important factor, and even limiting the crop productivity. The evaluation of the seedlings quality in the nursery can be a tool to identify their adequate development and if they are healthy with the maximum potential for survival after the transplant to the field (Amaral *et al*., 2011). Among the factors that interfere in the production of quality seedlings, the substrate and fertilization, can affect the seedlings growth and development, both in the nursery and in the implantation of field crops (Marcuzzo *et al.,* 2005).

Thus, it is fundamental to select the best substrate and fertilization for the optimal seedlings development, especially in the root system development. Although the coffee root system has its developmental characteristics linked primarily to plant genetics, other factors can also modify its spatial distribution, such as soil moisture and the availability of nutrients (Souza *et al*., 2013).

The search for new agricultural inputs is of great importance for agriculture and environmental sustainability (Evangelista *et al.,* 2013). Thus, it is important to know the effects of organic fertilizers on the availability of nutrients to plants, such as filter cake and lithothanmium. The filter cake is a residue composed of the mixture of ground sugarcane bagasse and sludge from the decantation, being derived from the sugar clarification process. In each ton of sugarcane, 30 kg to 40 kg of filter cake are produced. It is an organic compound rich in calcium, nitrogen and potassium, with a variable composition, depending on the sugarcane variety and its maturity (González *et al*., 2014). However, lithothanmium is a product derived from calcareous marine algae, which contains calcium carbonate, magnesium and even more than 20 microelements present in varying amounts, such as Fe, Mn, B, Ni, Cu, Zn, Mo, Se. The main characteristics that potentiate the performance of this product are attributed to the availability of the micronutrients that are adsorbed on the cell walls, being thus easily assimilable by plants and animals and the high porosity of the algae (> 40%), which provides a greater specific surface of action in soil (Dias, 2000).

The use of limestone algae (Lithothanmium), applied in a consortium with filter cake increases the availability of nutrients to the plants, due to the pH correction and the activation of the autotrophic bacteria development responsible for the nitrification process (Oliveira *et al.,* 2009). Studies carried out with this product have shown good results in the formation of yellow passion fruit and papaya seedlings (Mendoça *et al*., 2006; Hafle *et al*., 2009), 'Cleopatra' tangerine tree (Cruz *et al.,* 2008) and 'Swingle' citrumelo development (Araújo *et al.,* 2007). However, little is known about the application of Lithothanmium in the production of coffee seedlings.

The objective of this study was to evaluate the effect of two substrates (cattle manure and filter cake) interacting with four levels of organic fertilizer with lithothanmium in coffee seedlings production.

Materials and methods

The work was developed in the experimental area of the School of Agronomy, Federal University of Goiás (UFG), located in the geographical coordinates of 16º 35' South and 49º 16' West and 722 m elevation.

Two types of substrates were tested and five lithothamnium levels. Substrates: (i) conventional substrate composed by ravine soil classified as Oxisol, decomposed cattle manure and sand in proportions: 3:1:1 (weight/weight), adding 1 kg of superphosphate, 3 kg of potassium chloride and 2 kg of limestone per m^3 of substrate; (ii) alternative substrate composed by ravine soil, filter cake, in proportions of 4:1

(weight/weight), adding 1 kg of superphosphate, 3 kg of potassium chloride and 2 kg of limestone for m^3 of substrate.

The experimental design was completely randomized, with treatments in factorial arrangement 5 x 2, five lithothamnium levels (0, 1.75, 3.50, 5.25 and 7 kg m^{-3} of substrate), two substrates (x and y) and three replicates.

Soil and filter cake physical and chemical attributes are shown in Tables 1 and 2, respectively.

The seedlings were grown in plastic bags with 0.20 m high, 0.11 m wide and 0.07 m diameter, and were placed under a screen of 50% shading to avoid sunburn from excess insolation. The irrigation was performed daily to maintain soil moisture in field capacity.

Table 2. Chemical composition of the filter cake used in the experiment.

Chemical analysis					
Macronutrients	**Unit**	**Value**	**Micronutrient and others**	**Unit**	**Value**
P	kg ha^{-1}	0.28	Al	kg ha^{-1}	291.74
K	kg ha^{-1}	90	Zn	kg ha^{-1}	4.20
Ca	kg ha^{-1}	1.12	MO	%	16
Mg	kg ha^{-1}	72.94	Carbono	kg ha^{-1}	9.28
CTC	-	4.42	pH	-	6.40
Physical analysis					
Sand	g kg^{-1}	590	Silt	g kg^{-1}	90
Clay	g kg^{-1}	320	Bulk density	Mg m^{-3}	1.2

Table 2. Chemical composition of the filter cake used in the experiment.

Element	Unit	Value
N	g kg^{-1}	13.7
P	g kg^{-1}	15.7
K	g kg^{-1}	8.8
Ca	g kg^{-1}	14
Mg	g kg^{-1}	4.8
Cu	mg kg^{-1}	58
Mn	mg kg^{-1}	431
Zn	mg kg^{-1}	36.1
OM	kg^{-1}	29
C/N	-	12.03
pH	-	7.9

The plants morphological characteristics were evaluated monthly from July to November 2015: plant height, leaf number, stem diameter, substrate saline activity, and root length. After 120 days the plants were removed from the bags. Both plant height and root length were measured as soon as they were cut and separated.

Statistical analyzes were performed using SISVAR 3.01 software. Polynomial regression analyzes were applied for fertilization levels, and deriving the equations, the best fertilization level were determined to obtain the maximum growth of coffee seedlings. And, it was use Tukey test (P>0.05) to substrates.

Results and discussion

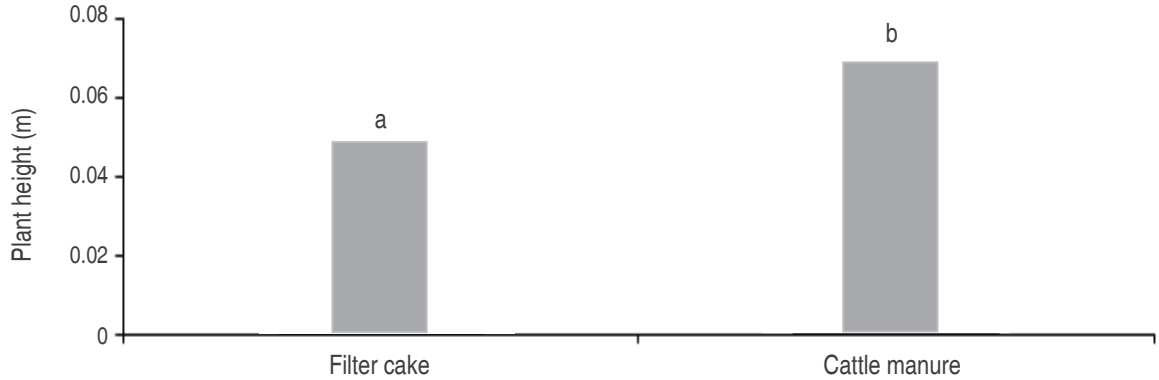
The results of the statistical analysis are presented in Table 3. It is observed that the plant height was influenced by both substrate and organic fertilization (lithothanmium) levels. The root length and saline soil activity were not influenced by the substrate, but by interaction substractes and lithothamnium levels. The stem diameter and number of leaves were not influenced by the treatments.

Coffee seedlings that were planted with cattle manure had higher plant height than with filter cake (Figure 1).

Table 3. Significant *P*-values of plant height (PlH), stem diameter (StD), number of leaves (NL), and root length (RL) of the coffee seedlings to different substrates and lithothamnium levels.

Variation source	*P*-value				
	PlH	StD	NL	RL	AS
Substrate	0.7176 *	0.00064 ^{ns}	0.54964 ^{ns}	3.5020 ^{ns}	0.0028 ^{ns}
Fertilization level	0.5325 *	0.07424 ^{ns}	1.24749 ^{ns}	10.075 *	0.3222 *
Substrate x F. Level	0.2878 *	0.08745 ^{ns}	0.64394 ^{ns}	9.6776 *	0.8623 *
Error	0.092	0.06009	3.17315	1.0567	0.1012
CV (%)	4.6	11.4	27.9	6.65	30.42

* Significant at 5% error probability; ^{ns} non-significant by the F test.



*Means followed by different letters differ by Tukey test at 5% error probability.

Figure 1. Plant height mean (PlH) of coffee seedlings developed in two substrates (filter cake and cattle manure)

Braun *et al*. (2009) found similar results.The authors report that the mean values of plant height produced with cattle manure were higher than with commercial substrate (Plantmax®) + soil. This result is probably due to the greater amount of organic material added in the substrate, which can improve infiltration, water retention, aeration, temperature and root penetration (Oliveira *et al.,* 2009).

A quadratic function was better fitted to lihothamnium levels in the cattle manure substrate, and linear function for the filter cake substrate (Figure2). The maximum root length of coffee seedlings was obtained in treatment with filter cake fertilized with 1.75 kg of lithothamnium per m^{-3} of substrate. The fertilization with levels higher than 1.75 kg of lithothamnium per m^{-3} of substrate reduced root length. While in cattle manure the maximum root length was with 5.25 kg m^{-3} of lithothanmium reducing at higher levels. According to Evangelista *et al*. (2015) high levels of lithothamnium cause negative effect on plant growth, due to the increase in pH of the soil and inhibition of nutrient absorption by plants. This can be

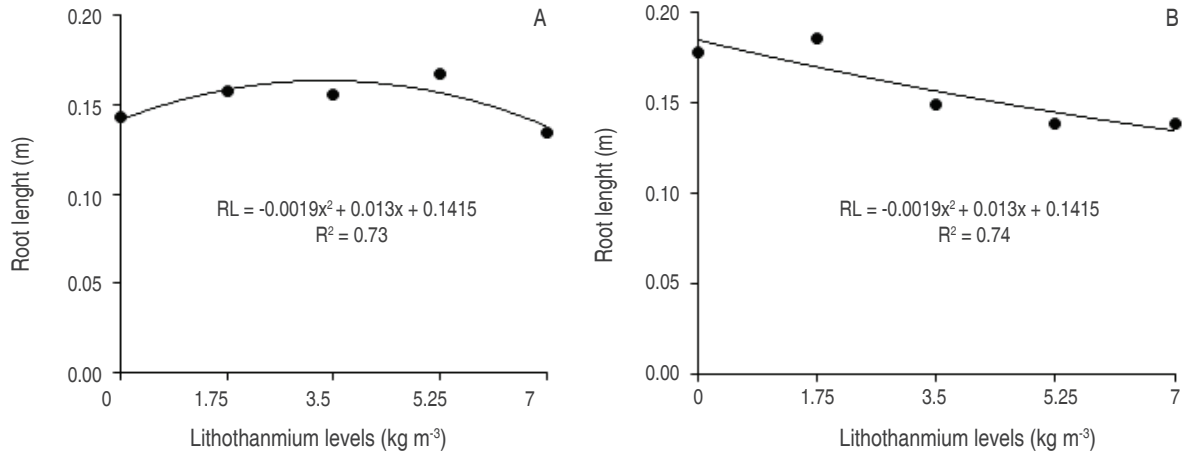


Figure 2. Regression analysis for the root length of coffee seedlings to different substrates and lithothamnium levels. A) Cattle manure. B) Filter cake.

justified by the possibility that the micronutrients present in lithothanmium, interacting with phosphorus and nitrogen in the filter cake, are given gradually by mineralization and attack of microorganisms in the soil, which causes greater efficiency by the plant (Santos *et al*., 2010).This may probably be due to smaller doses of lithothanmium, interacting with the phosphorus and the existing nitrogen in the filter cake, are slowly mineralized by the attack of microorganisms in the soil, which causes greater efficiency by the plant (Santos *et al*., 2010). It is suggested that the application of bioclastic granulate be made along with organic matter, once organic matter enhances chemical and physical properties of the soil (Damatto *et al*., 2006) in addition to having nutrients in its composition, which are best provided with the application of grained bioclastic (Days, 2000), favoring the coffee growth. Teixeira *et al*. (2015) and Mendoça *et al*. (2006) also evaluate the effect of application of lithothamnium levels and types of substrate on production of *Carica papaya* L. They found that high levels of this fertilizer had negative effects in the, and the authors attributed to a possible toxicity of the substrate.

CONCLUSIONS

The treatments produced significant effects on the initial development parameters of the coffee seedlings. The best results in plants grown were obtained in cattle manure substrate with 5.25 kg m^{-3} of lithothamnium, and decreased with levels higher than 1.75 kg m^{-3} of lithothanmium in filter cake substrate.

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Short article

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Organic acids to eliminate interference by phosphorus in the analysis of silicon by molecular absorption

Ácidos orgánicos para eliminación de interferencias por fósforo en el análisis de silicio por absorción molecular

doi: 10.15446/rfna.v70n2.64523

Enrique M. Combatt Caballero[1]*, Daniel Palacio Badel[2] and Manuel Palencia Luna[3]

ABSTRACT

One of the problems for quantifying the amount of silicon available by molecular absorption is the elimination of chemical interference caused by available phosphorus. The aim of this work was to evaluate different organic acids in eliminating the interference caused by phosphorus in the quantification of available silicon by molecular absorption. The experiments were conducted in the Soil laboratory of the College of Agricultural Sciences at the Universidad de Córdoba, Colombia. For this work, different acids such as tartaric, citric, oxalic and malic were evaluated at two concentrations (0.8 and 1.33 mol L^{-1}). Solutions containing silicon (1 mg L^{-1}) and six concentrations of phosphorus were prepared (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg L^{-1}). The quantification of silicon was conducted by molecular absorption spectrophotometry using a Perkin Elmer Lambda XLS + at 660 nm. The results were subjected to the LSD tests and contrasts using the R software (Development Core Team, version 3.2.2).The results indicated that the oxalic, citric and malic acids at both concentrations produced lower overestimation of silicon in the presence of the P concentrations 0.6, 0.8 and 1.0 mgL^{-1} than the tartaric acid, which is commonly used as a reference to remove the P interference.

Key words:
Quantification
Phosphomolybdate
Extraction methods
Mineral nutrition
Silicomolybdate

RESUMEN

Uno de los problemas para cuantificar la cantidad de silicio disponible por absorción molecular es la eliminación de la interferencia química causada por el P. El objetivo de este trabajo fue evaluar diferentes ácidos orgánicos para eliminar tal interferencia. Esta investigación fue realizada en el laboratorio de suelos y aguas de la Facultad de Ciencias Agrícolas de la Universidad de Córdoba, Colombia. Para el trabajo se evaluaron diferentes ácidos tales como tartárico (ácido de referencia), cítrico, oxálico y málico a dos concentraciones (0,8 y 1,33 mol L^{-1}). Para tal fin soluciones con silicio (1 mg L^{-1}) y seis concentraciones de P (0,0, 0,2, 0,4, 0,6, 0,8 y 1,0 mg L^{-1}) fueron preparadas. La cuantificación del silicio se realizó por espectrofotometría de absorción molecular con un Perkin Elmer Lambda XLS+ a 660 nm. Los resultados fueron sometidos a la pruebas de DMS y contrastes utilizando el software R (Development Core Team, versión 3.2.2). Los ácidos orgánicos oxálico, cítrico y málico a ambas concentraciones presentaron menor sobreestimación de silicio a dosis de P 0,6, 0,8 y 1,0 mg L^{-1}, que al ácido tartárico, el cual es utilizado como ácido de referencia para eliminar interferencias por P.

Palabras claves:
Cuantificación
Fosfomolibdato
Métodos de extracción
Nutrición mineral
Silicomolibdato

[1] Facultad de Ciencias Agrícolas. Universidad de Córdoba. AA 230002, Montería, Colombia.
[2] Facultad de Ciencias Químicas. Universidad de Concepción. Casilla 160-C, Concepción, Región del Biobío, Chile.
[3] Departamento de Química. Universidad del Valle. AA 25360, Cali, Colombia.
* Corresponding author <ecombatt@correo.unicordoba.edu.co>

Received: October 21, 2016; Accepted: March 20, 2017
Rev.Fac.Nac.Agron. 70(2): 70(2): 8183-8189. 2017. ISSN 0304-2847 / e-ISSN 2248-7026

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In analytical laboratories, where the nutritional elements are quantified, the determination of available silicon is affected by the P interference, making this the main problem in the determination of silicon by colorimetry. Atkins and Wilson (1926) reported that the P does not interfere with colorimetric determination of silicon, but Jimenez-Prieto and Silva (1998) explained that the main drawback in the determination of phosphorus and silicon are mutual interference of both species, which strongly influences the performance of analytical methods.

In the quantification of silicon and phosphorus concentrations in soil samples by colorimetric methods is used the reaction that occurs between phosphates and silicates with molybdate in an acidic medium, this forms the corresponding yellow heteropolymolybdates and occurs the subsequent reduction to molybdenum blue. But in this process there is interference between both species, which strongly influences the performance of analytical methods. This interference is because the ammonium molybdate is capable of forming phosphomolybdates complexes, which absorb the same wavelength in that the silicomolybdate.

Schwahtz (1942), Bunting (1944) and Case (1944) carried out studies to control the interference by P in quantifying silicon with organic acids oxalic, tartaric and citric by eliminating phosphomolybdate, to determine the silicon content in copper alloys and silicate rocks (Carlson and Banks (1952), Milton (1951) and Shell (1962) found that the silicomolybdate was stable with the addition of organic acids, only if the acid is added after the complex formation, also they found that it is possible to eliminate it after the complete formation of silicon-molybdate.

Jolles and Neurath (1988) developed the method of molybdate in the determination of inorganic silicon in water and soil; they additionally indicated that for the determination of silicon is necessary to employ the colorimetric method because it is very sensitive. This is based on the formation of silicomolybdate ($SiO_2.12MoO_3$), as shown in the following equation:

$$7Si(OH)_4 + 12\ H_6Mo_7O_{24}.4\ H_2O + 126H_2O <=> 7\ H_8Si(Mo_2O_7)6.28H_2O$$

In recent years studies about silicon chemistry have been intensified and the probable because this is an beneficial element for plants, several studies have showed benefits in crops, especially when plants are subject to different kind of stress, resistance to adverse factors (biotic and abiotic), which contribute to increase crop productivity specially grasses (Pulz *et al*., 2008); (Crusciol *et al*., 2009; Datnoff *et al*., 2001).

In South America and Colombia there are few studies that have been conducted to determine the real value of organic acids in phosphate removal during Si determination. Although many scientists have carried out research from the 1940´s to the 1980´s and determined that tartaric acid efficiently eliminated the interference caused by P in the analysis of silicon. Currently Korndorfer *et al*. (2004) eliminates this interference with tartaric acid.

Moreover, when it is need to determine and quantify the content of available silicon in soil and water other organic acids have showed similar or even greater efficiency in eliminating the P interference in soils containing high concentrations of phosphorus and silicon. Rocha *et al*. (2005), indicated that tropical soils are characterized by a high degree of weathering and low content of available phosphorus but due to the fact that currently the available silicon is quantified in soils with high content of P, different acids fulfill the same function of the tartaric acid. The aim of this study was to evaluate the effects of different organic acids to eliminate the interference caused by phosphorus in the chemical analysis of silicon.

MATERIALS AND METHODS

Four different organic acids (tartaric, citric, oxalic and malic acids) were used at two concentrations (0.8 and 1.33 mol L^{-1} as suggested by Korndorfer *et al*. (2004) and Freitas and Da Gloria (1976).

Six solutions containing silicon at 1 mg L^{-1} were prepared separately with six concentrations of phosphorus (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mg L^{-1}). The objective of that each experimental unit was to keep the same concentration of silicon with different concentration of P. This procedure was performed with four repetitions.

From these solutions were taken aliquots of 10 mL, which were added in 50 mL plastic cups. According to Korndörfer *et al*. (2004) to each aliquot was added 1 mL of sulfomolibdica solution for the complex formation

of α-molibdosilicato (yellow color), than occurrs after 5 min at pH 1.4-2.0.

After 10 min elapsed of adding the sulfomolibdica solution, 2 mL of organic acids at 1.33 mol L^{-1} were added to eliminate the P interference as proposed by Korndörfer *et al.* (2004). Also the concentration of organic acids 0.8 mol L^{-1} was added to an aliquot of 6 mL for each acid as suggested by Freitas and Da Gloria (1976).

During this process, it was necessary to wait 5 min for the corresponding reactions; that eliminate the fosfomolibdate complex, which are the causative agents of this interference. After this reaction 10 mL of ascorbic acid (3 g L^{-1}) were added to reduce molibdosilicate yellow into the molybdenum blue complex. One hour later readings were made at a spectrophotometer Perkin Elmer lambda XLS + at 660 nm.

The data were analyzed using analysis of variance, mean test, and contrast analysis and pairwise tests using the statistical software R (Development Core Team, version 3.2.2)

RESULTS AND DISCUSSION

It was found that organic acids did not eliminate the interference caused by P, especially as its concentration increased. The acids used followed the same trend of the reference acid (tartaric acid) proposed by Korndorfer *et al.* (2004) (Figure 1).

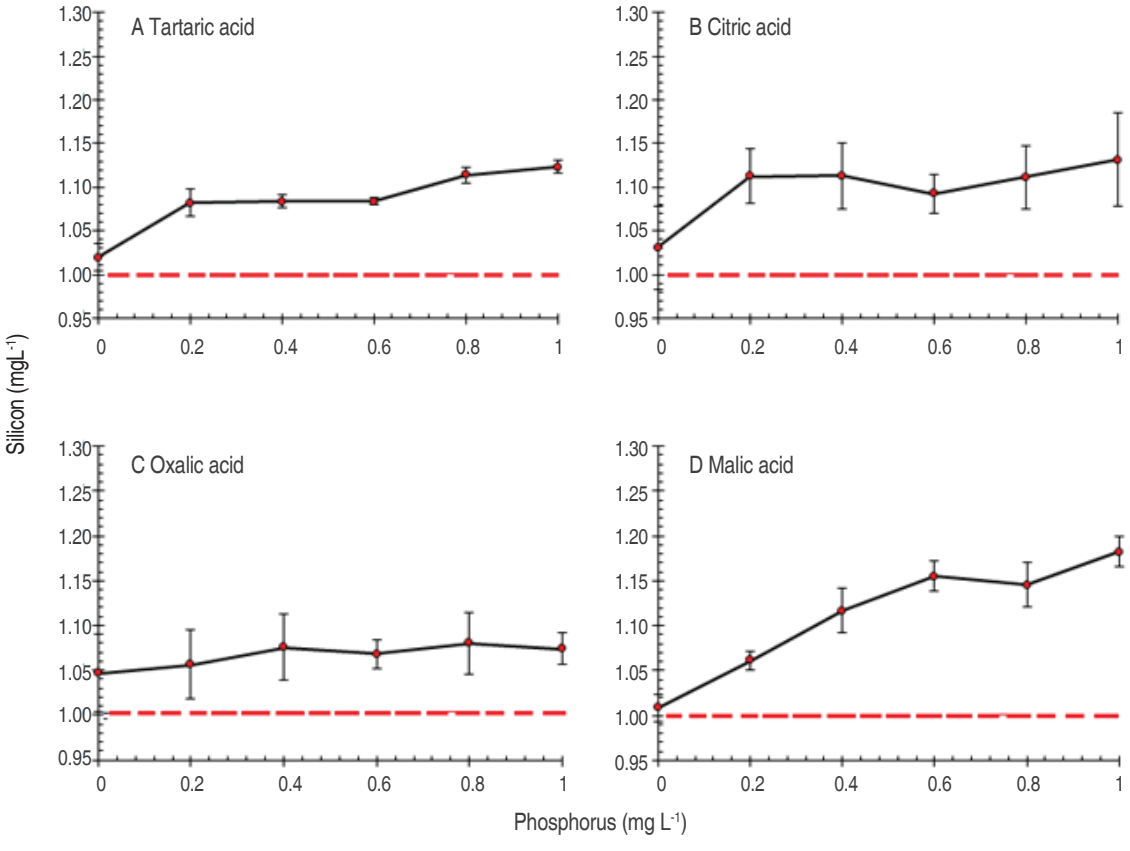

Figure 1. Silicon concentration (mg L^{-1}) determinated in the presence of increasing concentrations of phosphorus and four organic acids at 1.33 mol L^{-1}: A Tartaric acid, B citric acid, C oxalic acid and D malic acid.

The organic acids used at 1.33 mol L^{-1}, in average overestimated the silicon concentration of 1 mg L^{-1} by tartaric acid0.08 mg L^{-1}, oxalic acid by 0.07 mg L^{-1}, citric acid 0.1 mg L^{-1} and the malic acid 0.11 mg L^{-1}.

It should be noted that as the P concentration increased in the Si solution, the organic acids used at concentration of 1.33 mol L^{-1} overestimate the amount of Si initially applied. It is possible to consider that at this concentration the

acids did not destroy the α- phosphomolybdate complex; in this way, quantification the α- phosphomolybdate complex is quantified as α-molibdosilicato. Since these complexes have additive effects there are overestimations on the concentration of silicon.

According to Galhardo *et al*. (2000) the elimination of the P interference by organic acids can be explained by a ligand exchange reaction with phosphomolybdate complex. This produces phosphate and molibdo - acid (acid used for removing interference) while the silicomolybdate complex undergoes not change. This complex is relatively inert and does not allow that to occur a ligand exchange with the acid used.

It is clear the oxalic acid at 1.33 mol L^{-1} was the acid that presented the most effective to stabilize the silicon quantization, in despite of the increasing concentration. Dabin *et al*. (1968) recommended oxalic acid to remove phosphorus interference.

It was also found that at the concentration of 0.8 mg L^{-1} the tartaric acid overestimates the silicon concentration of 1 mg L^{-1} in 0.1 mg L^{-1} and the citric acid in 0.15 mg L^{-1}.

According to Carpenter *et al*. (1997) the rate of complex formation of molybdosilicate and molybdophosphate depends on pH and temperature, also the formation of

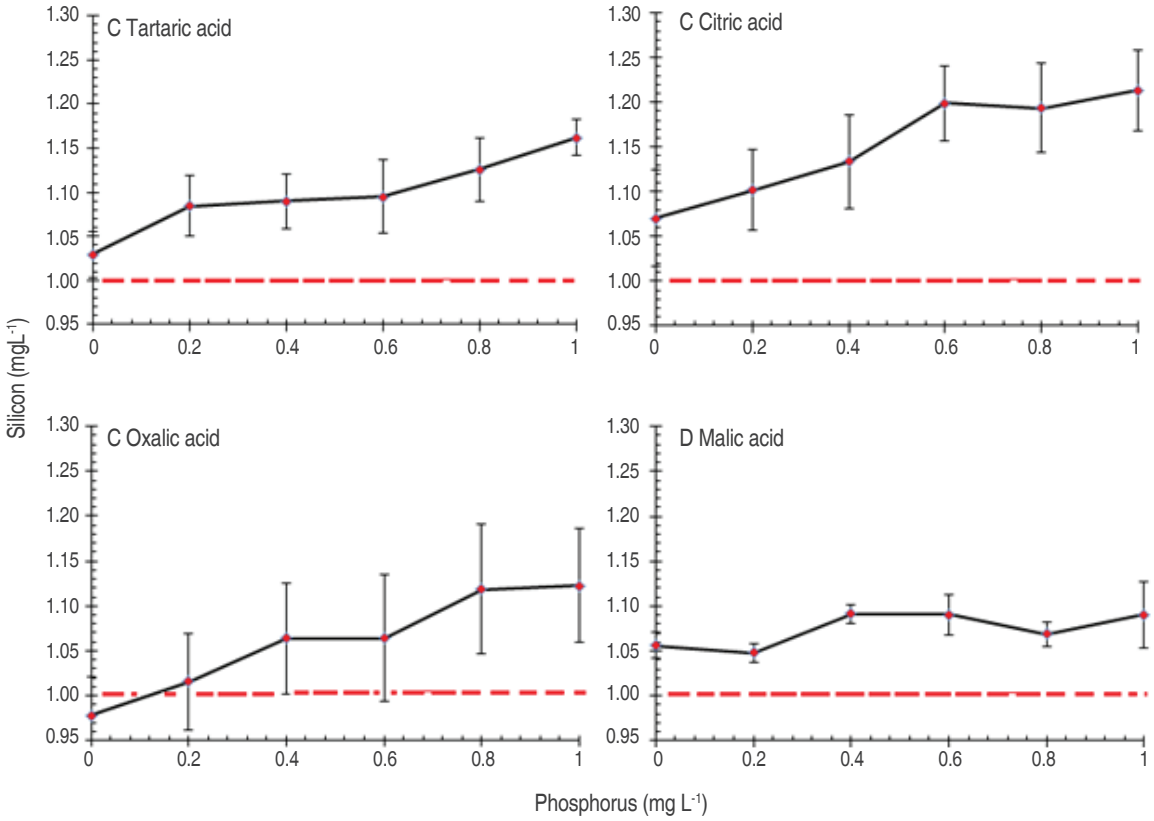

Figure 2. Silicon concentrations (mg L^{-1}) determined in the presence of increasing concentration of phosphorus and four organic acids (at 0.8 mg L^{-1}): A Tartaric acid, B citric acid, C oxalic acid, and D malic acid.

molybdophosphate is completed in less than 1 min and the rate increases in the more acidic solutions, while the formation of molybdosilicate would be slower (10 min), but it decreases in more acidic solutions.

At this concentration (0.8 mg L^{-1}), the acids with higher capacity of breaking the phosphomolybdate complex were oxalic and malic; in the presence of both acids the Si overestimated was 0.06 and 0.07 mg L^{-1}, being

the malic acid the most effective in the elimination of interference along the increasing gradient of P.

These results indicate the limited capacity of these acids to eliminate the interference by phosphorus at a concentration 1.33 mol L^{-1} in the quantification the available silicon (Table 1). According to Dabin (1968) and Mysumi and Tarutani (1961) the organic acids for eliminate interference phosphomolybdate are the oxalic and tartaric acids.

Similarly by comparing the results of the initial concentration of silicon (1 mg L^{-1}) with the silicon concentration obtained with organic acids at a concentration of 0.8 mol L^{-1} was determined than the values obtained the available silicon were equals statistically between the same dose of each acid, when compared to the data obtained with tartaric acid at this concentration. These results indicate that the organic acids used have the same ability to neutralize interference by phosphorus, that tartaric acid at concentrations of 0.8 and 1.33 mol L^{-1}.

These results may be explained by the ability of these organic acids have of ligand exchange with the phosphomolybdate complex, which is the causative agent of interference in the analysis of silicon. Galhardo *et al*. (2000) demonstrated than the quantification of phosphates and silicates by sequential injection, through spectrophotometric determination using the molybdenum blue chemical method, was possible eliminate interference caused by phosphates using oxalic acid.

Table 1. Test DMS for the silicon content with different methods, using different doses of phosphorus.

Methods (mol L^{-1})	Concentration					
	0.0	0.2	0.4	0.6	0.8	1.0
	(mg L^{-1})					
Tartaric acid 1.33	1.0197 abc	1.0818 ab	1.2424 a	1.0835 bc	1.1137 bc	1.1233 bcd
Citric acid 0.8	1.0698 a	1.1017 a	1.2290 a	1.1989 a	1.1933 a	1.1822a
Oxalic acid 0.8	0.9782 c	1.0156 b	1.0636 a	1.0642 c	1.1185 bc	1.1228 bcd
Tartaric acid 0.8	1.0298 abc	1.0892 a	1.0896 a	1.0948 bc	1.1259 abc	1.1622 abc
Oxalic acid 1.33	1.0468 ab	1.0564 ab	1.0755 a	1.0682 c	1.0803 bc	1.0742 d
Citric acid 1.33	1.0309 abc	1.1127 a	1.1129 a	1.0922 bc	1.1111 bc	1.1312 bcd
Malic acid 0.8	1.0560 ab	1.0477 ab	1.0914 a	1.0982 bc	1.0686 c	1.0904 cd
Malic acid 1.33	1.0086 bc	1.0611 ab	1.1169 a	1.0552 ab	1.1456 ab	1.1822 ab

Means followed with the same letter in the columns do not differ significantly according to DMS test at 5 % probability.

To verify the test results obtained with the least significant difference (LSD), orthogonal against (tartaric acid) were performed (Table 2).

It was determined that there were not significant differences among the effect of tartaric acid and C2, C3 , C4 , C5 , C6 and C7 at lower doses of 0.4 mg L^{-1} of phosphorus added. However, at the P concentrations of 0.6, 0.8, and 1.0 mg L^{-1} there were significance differences among tartaric acid and other organic acids used. This showed that the organic acids have greater ability to eliminate interference by phosphorus than the tartaric acid at a concentration the 1.33mol L^{-1} in the analysis of available silicon.

The malic acid can be used as an alternative to eliminate interferences caused by phosphorus. Chalmers and Sinclair (1966), indicate that the use of tartaric acid, to mask phosphate interference in determining silicate reduces the sensitivity of the method. This is likely due to a marked change in the extinction coefficient of phosphomolybdate formed and the electrochemestry´s factors that determine its response should be very different.

This results should be corroborated to check the use of malic acid in quantifying silicon by molecular adsorption. In addition, the malic acid is more economical compared to tartaric acid.

Table 2. Contrasts averages for the silicon content of eight organic acids and six doses phosphorus in removing phosphorus chemical interference.

Contrasts	Methods	Concentration					
		0.0	0.2	0.4	0.6	0.8	1.0
		P (mg L^{-1})					
C1	M1 vs M2	-0.0501 ^{NS}	-0.0198 ^{NS}	0.0133 ^{NS}	-0.1154 *	-0.0796 *	-0.0905*
C2	M1 vs M3	0.0415 ^{NS}	0.0662 ^{NS}	0.1787 ^{NS}	0.0192 ^{NS}	-0.0047 ^{NS}	0.0004 ^{NS}
C3	M1 vs M4	-0.0101 ^{NS}	-0.0024 ^{NS}	0.1528 ^{NS}	-0.0113 ^{NS}	-0.0122 ^{NS}	-0.0389 ^{NS}
C4	M1 vs M5	-0.0271 ^{NS}	0.0254 ^{NS}	0.1669 ^{NS}	0.0152 ^{NS}	0.0334 ^{NS}	0.0490 ^{NS}
C5	M1 vs M6	-0.0111 ^{NS}	-0.0308 ^{NS}	0.1294 ^{NS}	-0.0087 ^{NS}	0.0025 ^{NS}	-0.0079 ^{NS}
C6	M1 vs M7	-0.0363 ^{NS}	0.0341 ^{NS}	0.1509 ^{NS}	-0.0067 ^{NS}	0.0451 ^{NS}	0.0328 ^{NS}
C7	M1 vs M8	0.0111 ^{NS}	0.0207 ^{NS}	0.1255 ^{NS}	-0.0717 ^{NS}	-0.0319 ^{NS}	-0.0589 ^{NS}

C1: tartaric acid 200 g L^{-1} (1.33 M) VS 0.8M citric acid C2: tartaric acid 200 g L^{-1} (1.33 M) VS 0.8M oxalic acid, C3: tartaric acid 200 g L^{-1} (1.33 M) VS tartaric acid 0.8 M C4: tartaric acid 200 g L^{-1} (1.33 M) C5: oxalic acid1.33 M VS tartaric acid 200 g L^{-1} (1.33 H) VS citric acid 1.33 M C6: tartaric acid 200 g L^{-1} (1.33 M) VS 0.8 M malic acid; C7: tartaric acid 200 g L^{-1} (1.33) 1.33 M VS malic acid; NS: not significant,
* Significant at 5% ** significant at 1 %.

CONCLUSIONS

The malic acid at a concentration of 0.8 and oxalic acid at 1.33 mol L^{-1} can be used to eliminate the interference of phosphorus in the quantification available silicon.

The organic acids had less overestimation of available silicon when there were concentration of P larger than 0.6, 0.8, and 1.0 mg L^{-1}, than the tartaric acid used as reference acid.

ACKNOWLEDGEMENTS

The authors are grateful for the funding and support from the staff in the Soils Laboratory ascribed to the Faculty of Agricultural Sciences the Universidad de Córdoba, Colombia.

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Bioconcentration of chlorpyrifos in roots and foliage of plants of *Cenchrus clandestinus* (Hochst. ex chiov.) morrone, cultured in green house

Bioconcentración de chlorpyrifos en raíces y follaje del pasto *Cenchrus clandestinus* (Hochst. ex chiov.) morrone, cultivado en inverdadero

doi:

Sara María Márquez Giron[1], Néstor Jaime Aguirre Ramirez[2] and Jaime Alberto Palacio Baena[2]

ABSTRACT

Keywords:
Insecticide
Systemic
Application
Bioaccumulation
Grass

The bio-concentration of the insecticide chlorpyrifos in grass *Cenchrus clandestinus* cultivated in green house was determined. The pesticide was applied in the culture solution and we carried out the monitoring of the concentration of the pesticide in foliage and root tissues during 72 h of exposure to the nutrient solution with 96, 192 and 288 mg L^{-1} of chlorpyrifos. Tissue samples were taken at 4, 24, 48 and 72 h. Removal of chlorpyrifos of foliage and grass root used the MI 48640 with a sensitivity of 0.01 ppm and the quantification was carried out by gas chromatography. During the study, it was possible to observe that chlorpyrifos is transported through the vascular system of plants in grass *C. clandestinus* in hydroponic cultivation, from the root to the foliage. In addition, it showed an increasing bioaccumulation in the tissues of roots and foliage. Bioaccumulation of chlorpyrifos was higher in the root 39.4 µg g^{-1} than in foliage 1.1 µg g^{-1}. Therefore, the use of this insecticide on livestock systems from high tropical areas represents a risk for cattle and other members of the dairy food chain.

RESUMEN

Palabras claves:
Insecticida
Sistémico
Aplicación
Bioacumulación
Pasto

Se determinó la bioconcentración del insecticida clorpirifos en plantas de pasto *Cenchrus clandestinus* cultivadas hidropónicamente, el pesticida fue aplicado en la solución de cultivo, se realizó el seguimiento de la concentración del pesticida en los tejidos del follaje y de la raíz, durante 72 h de exposición a la solución nutritiva con 96, 192 y 288 mg L^{-1} de clorpirifos. Los muestreos en tejidos se realizaron a las 4, 24, 48 y 72 h. Para la extracción del clorpirifos del follaje y raíz del pasto se empleó el método MI 48640 con una sensibilidad de 0,01 ppm y la cuantificación se realizó por cromatografía de gases. Durante el estudio se evidenció que el clorpirifos se transporta a través del sistema vascular de plantas de *C. clandestinus* en cultivo hidropónico, desde la raíz hasta el follaje. Además, se evidenció bioacumulación creciente en los tejidos de raíz y follaje. La bioacumulación del clorpirifos fue mayor en la raíz 39,4 µg g^{-1} que en el follaje 1,1 µg g^{-1}. Por lo anterior, la utilización de este insecticida en los sistemas ganaderos de trópico alto representa un riesgo para los bovinos y para los demás integrantes de la cadena alimenticia láctea.

[1] Facultad de Ciencias Agrarias. Universidad de Antioquia. AA 1226, Medellín, Colombia.
[2] Facultad de Ingeniería. Universidad de Antioquia. AA 1226, Medellín, Colombia.
* Corresponding author <saramariamarquezg@gmail.com>

Received: April 8, 2016; Accepted: February 10, 2017
Rev.Fac.Nac.Agron. 70(2): 8191-8201. 2017
ISSN 0304-2847 / e-ISSN 2248-7026

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The high demand for pesticides in most regions of the world is caused by intensive systems of farming that have been implemented based on the establishment of monocultures, which according to Altieri and Nicholls (2007), have involved the simplification of biodiversity. Therefore, farms have become artificial ecosystems, highly dependent on the contribution of agro-chemicals that unbalance even more the system. Plants grown under these intensive systems do not have enough ecological defense mechanisms to tolerate the impact of pests and diseases. Therefore, excessive use of chemical plant protection products is necessary (Chará and Giraldo, 2011; Cruz *et al.,* 2006).

In the area dedicated to the breeding milk located in the municipality of San Pedro de Los Milagros predominates an intensive production system with herds grazing and intensive cultivation of pastures, mostly *C. clandestinus*, which has been based on monoculture with high loads of organic fertilizer called porquinaza and chemical fertilization, with rest periods of 45 to 60 days (Quirós *et al*., 1997).

In these production systems Marquez (2001) detected the presence of chlorpyrifos in the foliage of *C. clandestinus*, even in the day 63 after applying the product, whose withdrawal time is 20 - 30 days, ICA sale registration 787. The processes of sampling pesticides by plants may correspond to an active transport in the absorption by the roots or a passive process as a result of direct contact between the biota and pesticides. Depending on the properties of pesticides and the characteristics of the biota, absorbed pesticide can be degraded, accumulated on the inside of the biota, or transferred to other agencies within the food chain. The pesticides sampling from plants is the first step in the process of bio-magnification, which can be made from contaminated soil or from the air. Tree leaves are particularly exposed to air pollution (Trapp and Matthies, 1998).

The accumulation of pesticides remains within the body of any organism is highly influenced by the body lipid content and the lipid affinity of the pesticide. Although, actually, the content of lipids in the tissues of the plants is not very significant, some plant species have shown high bioaccumulation factors (Zacharia *et al*., 2010). The anatomy and the physiology of vascular tissues define routes of pesticides to plants inside. The compounds enter by the root and move the stem through the xylem apoplastic system, but must also have the ability to enter to the symplast (Trapp and Mc Farlane, 1995).

Chlorpyrifos, O, O-diethyl O-3, 5, 6 trichloro-2-pyridyl phosphorothioate (Royal Society of Chemistry, 1987) is an insecticide, acaricide with degree II of toxicity. Figure 1 shows the structural formula and Table 1 presents the main physical and chemical characteristics (European Commission Health and Consumer Protection Directorate-General (2005) and British Crop Production Council (2006). It is a non-systemic compound, widely used for the control of agricultural pests and in veterinary medicine for the control of ticks and lice (Castelli *et al*., 2007).

Iannacone *et al*. (2000), assessed the duckweed *L. minor* bioassays semi-static ecotoxicological, with the pesticides, chlorpyrifos and lindane, dissolved in the nutrient solution.

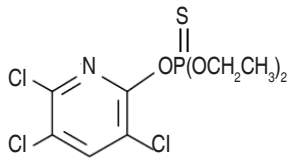

Figure 1. Structural formula of chlorpyrifos.

Table 1. Physical-chemical characteristics of chlorpyrifos.

Property	Valuation
Molecular formula	$C_9H_{11}Cl_3NO_3PS$
Molecular mass	350.6
Melting point	41 - 42 °C
Boiling point	170 - 180 °C
Appearance	Amber to white crystalline solid, with light smell mercaptan.
Density relative	1.51 (98.1)

The test duration was 48 h. The sub lethal effects were evaluated: formation of new leaves, chlorosis (50% of pigment loss), necrosis (50% of dead tissue) and rupture of colonies. As result, for chlorpyrifos, four sub lethal effects were found in the following sequence, in order of decreasing ecotoxicity: breakdown of colonies, formation of new leaves, chlorosis and necrosis.

While studying the distribution of non-ionized chemicals in barley plants hydroponically cultivated, through the monitoring of sampling by the roots from a solution, Briggs *et al*. (1983) found that the concentrations of the ortometilcarbamoiloximas and the phenylureas replaced on the basement and central sections of the plant reach steady state between 24 and 48 h and that the concentrations of pesticides in leaves increased from 72 to 96 h.

Hsu *et al*. (1990) studied the root samples and the transport of Cinmetilina and compounds associated with xylem in soybean plants hydroponically cultivated and cut above the neck, using the technique of the pressure chamber. These authors found a non-linear relationship between partition coefficient octanol-water (K_{OW}) of substances and the concentration factor in the flow of perspiration at the base of the stem (TSCF). The highest values of the TSCF ranged from 0.6 to 0.8 and corresponded to the compounds with values of log K_{OW} between 2.5 and 3.5.

This research was carried out with the purpose of giving answer to the following question: does chlorpyrifos bio-concentrate in the tissues of roots and foliage of *C. clandestinus*, under hydroponic conditions? Also, it was carried out to refute or endorse the following hypothesis: "If chlorpyrifos can be taken through the roots and transported from there through the vascular system to the foliage of *C. clandestinus*, then, it is possible to detect the presence of this substance in significant concentrations in the root and the foliage." The objective of this work was to establish the potential for bioaccumulation in the root and the foliage of *C. clandestinus*, cultivated hydroponically.

MATERIALS AND METHODS

Study area

The municipality of San Pedro de Los Milagros, with an area of 229 km^2, located in the Sub-region of North Highlands, 6°27´19´´ North latitude and 75°37´40´´ of Western length in the Department of Antioquia (Colombia), has an average height above the sea of 2475 m and an average temperature of 14 °C (Gobernación de Antioquia, 2002).

The cespedones of grass *C. clandestinus* for experimentation were taken from La Montaña farm, property of the University of Antioquia and located in the municipality of San Pedro de Los Milagros, sidewalk Monte Redondo. In this farm, a specialized dairy operates. They work with Holstein cattle, pure and Blanco Orejinegro x Holstein. The annual average rainfall is 2500 mm, the altitude is 2360 m, the average temperature is 16 °C, in an area of low life mountainous rain forest (Bhmp). It has an area of 33 ha, of which 28 ha are established with Kikuyu grass pastures (*C. clandestinus*), on a flat relief or topography slightly undulating with loose natural drainage. These cespedones were obtained from a pasture in which pesticide has not been applied for 12 years for the control of scenic collaria and regularly treated with organic fertilizer.

Test for bio-concentration

In order to establish the kinetics of the process of absorption – bio-concentration of chlorpyrifos in the *C. clandestinus*- the monitoring of the concentration of chlorpyrifos in foliage and root tissues was carried out during 72 h of exposure to the nutrient solution with 96, 192 and 288 mg L^{-1} of chlorpyrifos. Tissue samples were made at 4, 24, 48 and 72 h.

Experimental design

In this study, a split-plot design was used. This design was applicable to experimental conditions where the assignment of treatments of a factor to main plots arranged in a completely randomized design is involved. In addition, the company introduced the time as an additional factor. The main plots correspond to a group of plastic trays with hydroponic cultivation of Kikuyu (*C. clandestinus*). The Lorsban was applied to each nutrient solution culture only once. This substance is a broad-spectrum organophosphorus insecticide with a chlorpyrifos concentration of 44.50%. From each group of trays, one was retired at 4, 24, 48 and 72 h (Figure 2). For each of the treatments, 3 vessels were available with 20 groups of plants developed from cespedones.

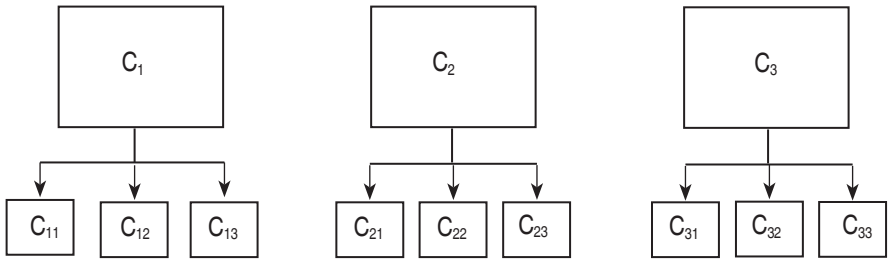

Figure 2. Experimental test design of chlorpyrifos accumulation in plants of the Kikuyo (*C. clandestinus*).

Field work

The cespedones of grass *C. clandestinus*, of similar size and weight, were planted in plastic containers of 0.60 m long, a 0.40 m wide and 0.15 m deep, covered up, internally, with aluminum foil. In vessels, substrate quartz grit, previously washed, was used. The nutrients were applied periodically in a specific nutrient solution for hydroponics. The environmental conditions in the greenhouse (Figure 3), during the 60 days of cultivation, were similar to the characteristic temperature of the North zone of the Department of Antioquia (16 ± 2 °C) and a photoperiod of 12 h.

The application of the pesticide was calculated from the dosage recommended by the house producer (400 $cm^3\ ha^{-1}$). The treatments were defined with Lorsban,

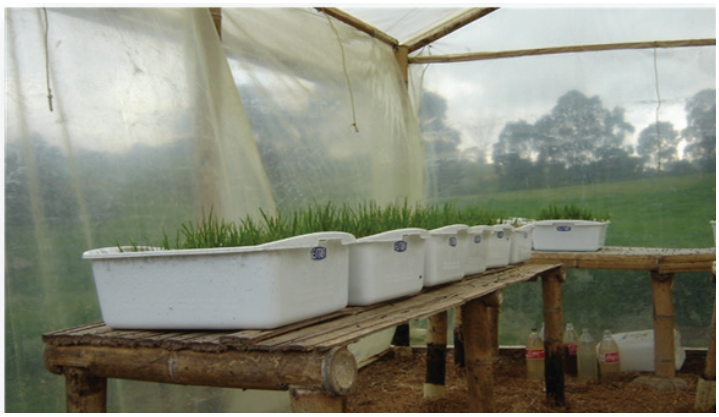

Figure 3. The hydroponic greenhouse.

corresponding to concentrations of nutrient solutions of cultivation of 200, 400 and 600 $cm^3\ ha^{-1}$ and a non-pesticide control. In this way, it was possible to work with the recommended dose (400 $cm^3\ ha^{-1}$), a lower dose (200 $cm^3\ ha^{-1}$) and upper one (600 $cm^3\ ha^{-1}$). Treatment number 1 (Lorsban 4 EC 200 $cm^3\ ha^{-1}$) corresponded to a concentration of the nutrient solution of 96 mg L^{-1} of chlorpyrifos, the number 2 to a concentration of the nutrient solution of 192 mg L^{-1} and the number 3 to a concentration of the nutrient solution of 288 mg L^{-1} of chlorpyrifos.

Sampling

Samples for the assessment of concentrations of chlorpyrifos in the foliage were taken under the abduction unit system (tray with hydroponic cultivation of grass) sampling without replacement of the solution. For the analysis of chlorpyrifos in foliage samples were extracted at 4, 24, 48 and 72 h after the application of chlorpyrifos to the nutrient solution, with three replicates per treatment and per sampling frequency. The cespedones, in each deck, received the following procedure:

- Cutting the foliage and storage in glass containers previously washed with soap and chromo solution.
- Separation of the cespedon and the grit attached to the roots.

– Sampling of root and storage in containers washed with soap and solutions.

Chemical analysis and chromatographic

The wet weight of the foliage and roots was determined for each sample of plants. From each sample, at least three replicas were analyzed, a rich sample of each array, soil, foliage, root or water of known concentration subject to the same procedure, with calibration curve for each assay. The final result was reported with statistical values using the t Student tabbed for n-1 degrees of freedom and a confidence level of 95.

Removal of chlorpyrifos of foliage and grass root used the MI 48640 method proposed by Dow Chemical (NTIS, 1987) with a sensitivity of 0.01 ppm. Acetonitrile was used for the initial extraction and for the final extraction acetone and hexane were used. The sample was homogenized in a food processor and then, it was extracted with ethyl acetate using ultrasound and a sonic disruptor, filtered or strained through a column of anhydrous sodium sulfate to remove excess water, the removal procedure was performed twice. Subsequently, the sample focused on the rotary evaporator. The concentrated sample is reconstituted quantitatively with ethyl acetate to be read in the gas chromatograph Hewlett Packard model 1800A, with auto sampler and mass detector.

With the information obtained, curves of bioaccumulation of chlorpyrifos for each treatment were developed. In addition, it was possible to search the mathematical model that best represents each treatment, using the Matlab and Stat graphics plus software version 5.1, taking into account the model of a compartment in the case of the root and foliage.

Statistical analysis

The arithmetic mean, median, standard deviation, variance, coefficient of variation and the range were defined according to the results of the concentration of chlorpyrifos in the foliage and the root of grass *C. clandestinus*.

In order to determine temporal trends of concentration of chlorpyrifos ($\mu g\ g^{-1}$) in foliage and root, graphics were constructed to link these two variables with the time in h. This same procedure was applied to the variable mass per unit area.

An analysis of non-parametric variance corresponding to the Kruskal Wallis test was performed to compare the bio-concentration of chlorpyrifos between arrays. Stat graphics plus, version 5.1 package was used for statistical analyses.

RESULTS AND DISCUSSION

Although, chlorpyrifos is classified as a non-systemic product, it was detected in the tissues of root and foliage (stem and root), being higher for the root. It is possible to observe the results corresponding to the concentration of chlorpyrifos in the two tissues of the grass, the concentrations of chlorpyrifos in the solution of cultivation, the sampling time and each repetition.

According to Marquez (2001), the concentration of chlorpyrifos in the foliage of plants of grass *C. clandestinus* cultivated in bag increased after a single application of the pesticide until day 35. Putnam *et al*. (2003) studied the persistence, distribution and degradation of chlorpyrifos in a commercial cultivation of blueberry developed in wetland and it was detected in the fruit harvested (62 d after application), but no metabolites were found.

It was evident that chlorpyrifos is a systemic compound and that is transported through the vascular system of plants in grass *C. clandestinus* in hydroponic cultivation, from the root to the foliage. Also, it showed bioaccumulation in tissue of the root and foliage grass *C. clandestinus* in hydroponic cultivation. Bioaccumulation of chlorpyrifos was higher in the root than in the foliage. This behavior was equal to the observed by Sashwati and Osmo (1994) the aquatic macrophyte *Eichhornia crassipes*, aquatic plant used for wastewater treatment. The accumulation in the roots and the leaves was higher during the first 12 h of exposure and then, it decreased gradually. Also, it was possible to find a higher bio-concentration in roots than in leaves (Bernal, 1991).

In Table 2 are the averages of the results of the bio-concentration of chlorpyrifos in the root of grass *C. clandestinus*. In all the treatments and the sampling periods, chlorpyrifos in the root was detected. It was possible to register an increase in concentrations over time exposure. In addition, a tendency to increase the concentration of the pesticide in the root is evident to increase the concentration in the nutrient solution.

Table 2. Concentration of chlorpyrifos in root of the hydroponic cultivation of *C. clandestinus.*

Dosis (mg L^{-1})	Sampling time (hours)	Concentration Average (µg g^{-1})	Standard deviation
96	4	1.986	0.645
96	24	3.484	0.406
96	48	5.574	0.651
96	72	13.482	0.778
192	4	3.309	0.563
192	24	6.003	3.006
192	48	8.143	1.855
192	72	18.001	2.532
288	4	9.896	2.202
288	24	12.581	1.277
288	48	19.118	2.322
288	72	39.412	7.790

The following results were obtained from the statistical analysis (Table 3): in this case, the value of the standardized coefficient of asymmetry and the standardized coefficient of Kurtosis are within the expected range for the data of a normal distribution, a median of 9337 µg g^{-1}, a variance of 106.885 and a range of 46.237 µg g^{-1}, with a minimum of 1.38. The p-value of the non-parametric estimator of Kruskal-Wallis test (F) was less than 0.055, indicating no statistically significant differences between the concentrations of chlorpyrifos in the root at different doses.

Within the analysis of variance components, it was possible to find that the factor that contributes to the maximum variance is the exposure time, its contribution represents 63.848 of the total variation in the concentration of chlorpyrifos in the tissue root and the application rate represents the 36.15.

Table 3. Statistical values of central tendency and variation for the concentration of chlorpyrifos in root.

Statistical Parameter	Value
Frequency	36.000
Median µg g^{-1}	9.337
Variance	106.885
Typical Deviation	10.338
Minimum µg g^{-1}	1.380
Maximum µg g^{-1}	47.610
Range µg g^{-1}	46.237
Classified Asymmetry	4.559
Classified Kurtosis	4.820

Model of the absorption of chlorpyrifos in root

Mathematical models obtained for the absorption of chlorpyrifos in the tissue root taking into account as factors, the concentration of chlorpyrifos in the nutrient solution, sampling time subsequent to the application of pesticide and its respective replays and statistical validation are as follows (Figure 4).

The regression analysis shows the results of the adjustment to the linear model (Table 4), to describe the relationship between the concentration of chlorpyrifos in soil (Cs) of the crop in the bag and time (t) in days after the application, to different doses applied, as well as the confidence level obtained from the value of the p from

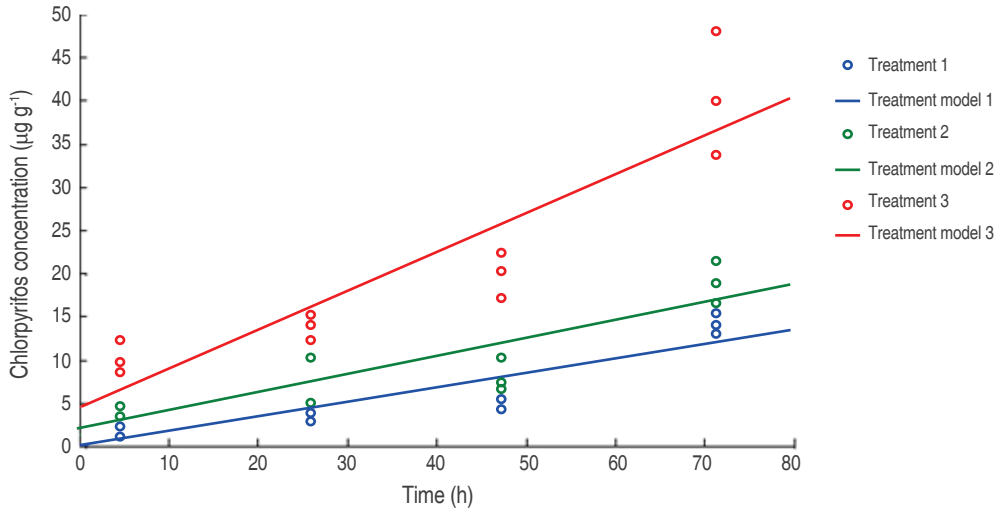

Figure 4. Mathematical models of the absorption of chlorpyrifos in root.

Table 4. Mathematical models of bio-concentration of chlorpyrifos in root.

Dose (cm^3 ha^{-1})	Model of residuality of chlorpyrifos in root (µg g^{-1})	Confidence level (%)	R^2 (%)	Correlation coefficient	Standard error
96	C_s = 0.1306 + 0.1621 * t	99	86.05	0.9276	1.8253
162	C_s = 1.2997 + 0.2044 * t	99	80.12	0.8951	2.8464
288	C_s = 4.6274 + 0.4222 * t	99	79.67	0.8926	5.9627

the ANOVA which was 99 for all doses. The R^2 that indicates the percentage in which the model explain the variability of Cs fluctuated between 79.67 and 86.05, the correlation coefficient between variables that was in the range of 0.8525 to 0.9391 standard error which was less than 0.4287 in all cases.

In *Eichhornia crassipes*, Sashwati and Osmo (1994) found that the chemical compound was transported to the leaves through the conductive tissues and that the xenobiotic is accumulated, but at a lower rate in the roots. In order to study the transport of not ionized chemicals from the root to the leaves in a hydroponic growing of barley, Briggs *et al*. (1983) found that the concentrations of several pesticides in the basal and central sections of plants become constant from the 24 to the 48 h and concentrations in leaves increased from 72 to 96 h. In this study, the chlorpyrifos showed a trend to stabilize in the foliage of the *C. clandestinus* 48 h after the pesticide was applied.

Table 5 shows the results chlorpyrifos averages in foliage grass *C. clandestinus* hydroponically cultivated under greenhouse. In all treatments and periods of exposure, the presence of chlorpyrifos in the foliage of the grass was detected. During the treatments, an increase in concentrations of the insecticide over time exposure was registered . In addition, it was possible to observe a tendency to increase the concentration of the pesticide in the foliage by increasing the concentration of the same in the nutrient solution. The following results were obtained from statistical analysis (Table 6).

In this case, the value of the standardized coefficient of asymmetry and the standardized coefficient of Kurtosis are within the expected range for the data of a normal distribution, a median of 0.387 µg g^{-1}, a variance of 0.099 and a range of 1.18 µg g^{-1}, with a minimum of 0.97.

The p-value of the estimator non-parametric Kruskal-Wallis test (F) was less than 0.05, indicating no statistically

Table 5. Bio-concentration of chlorpyrifos in foliage of the hydroponic cultivation of *C. clandestinus*.

Dosis (mg L^{-1})	Sampling time (hours)	Concentration average (µg g^{-1})	Standard deviation
96	4	0.140	0.053
96	24	0.270	0.055
96	48	0.387	0.049
96	72	0.491	0.017
192	4	0.233	0.038
192	24	0.349	0.041
192	48	0.499	0.081
192	72	1.049	0.198
288	4	0.316	0.056
288	24	0.382	0.017
288	48	0.699	0.028
288	72	1.149	0.121

Table 6. Statistical values of central tendency and variation for the concentration of chlorpyrifos in foliage.

Statistical parameters	Value
Frequency	36
Median µg g^{-1}	0.387
Variance	0.098
Typical Deviation	0.313
Mínimum µg g^{-1}	0.097
Maximum µg g^{-1}	1.277
Range µg g^{-1}	1.180
Typified Asymmetry	3.052
Typified Kurtosis	0.924

significant differences between the concentrations of chlorpyrifos in the foliage at different doses.

Within the analysis of the components of variance, it was possible to find that the factor that contributes to the maximum variance is the exposure time. Its contribution represents 85.74 of total variation in the concentration of chlorpyrifos in the foliage and the application rate represents the 14.26.

Model of the absorption of chlorpyrifos in foliage

The mathematical models obtained for the absorption of chlorpyrifos in the tissues that make up the foliage taking into account, as factors, the concentration of chlorpyrifos in the nutrient solution, sampling time subsequent to the application of pesticide and its respective replays and statistical validation, are as follows (Figure 5).

The regression analysis shows the results of the adjustment to the linear model (Table 7), to describe the relationship between the concentration of chlorpyrifos in soil (Cs) of the crop in the bag and time (t) in days after the application, to different doses applied, as well as the confidence level obtained from the value of p of the ANOVA which was 99 for all doses. The R^2 that indicates the percentage in which the model explain the variability of Cs fluctuated between 81.47 and 91.62. The correlation coefficient between variables was in the range of 0.9026 to 0.95.72 and the standard error was minor than 0.1535 in all cases.

With the highest application of insecticide, an increase of concentration in plant tissue of grass *C. clandestinus* with a higher level of exposure was found. Lower chlorpyrifos concentrations were reported in the foliage of grass

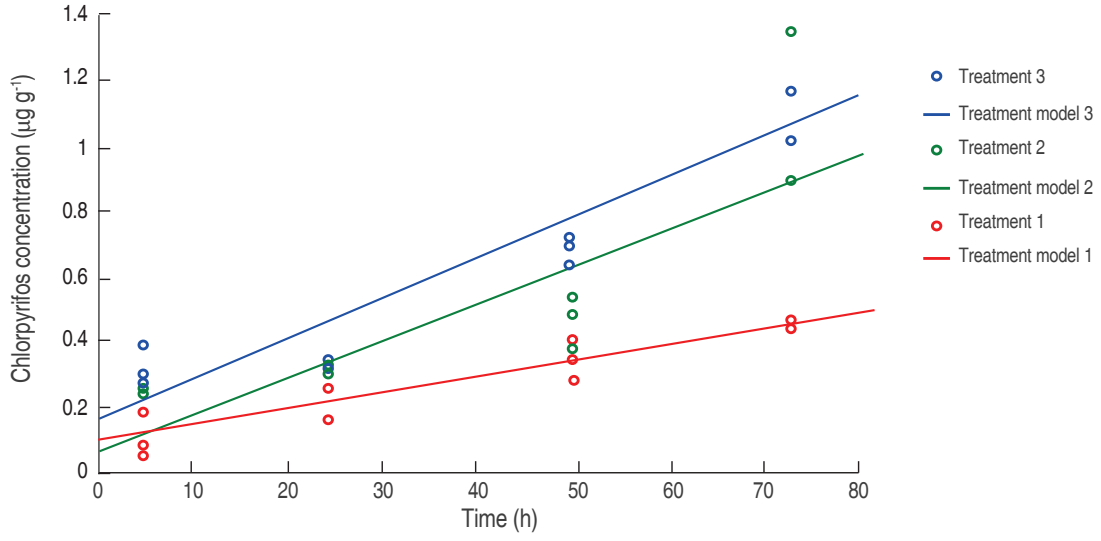

Figure 5. Mathematical models of the absorption of chlorpyrifos in foliage of *C. clandestinus.*

Table 7. Mathematical models of bio-concentration of chlorpyrifos in foliage *C. clandestinus.*

Dose ($cm^3 ha^{-1}$)	Model of residuality of chlorpyrifos in foliage ($\mu g\ g^{-1}$)	Confidence level (%)	R^2 (%)	Correlation coefficient	Standard error
96	$C_s = 0.1325 + 0.0051 * t$	99	91.62	0.9572	0.0432
162	$C_s = 0.1062 + 0.0115 * t$	99	81.47	0.9026	0.1535
288	$C_s = 0.1744 + 0.0124 * t$	99	90.89	0.9534	0.1104

C. clandestinus. Stabilization in the concentrations at 48 h after application, coincides with what was reported by Sashwati and Osmo (1994) since, during the initial phase of the xenobiotic sampling, the plant is fast and reaches a nearly steady state after 24 to 48 h of exposure to the xenobiotic. However, Briggs *et al.* (1983) argue that they need between 72 and 96 h for the xenobiotic concentrations increase in the leaves.

Analyzing the trends of the graphs of the concentrations of the product on the foliage, it is possible to demonstrate and prove the issues pointed out by Briggs *et al*. (1983) when they say that any compound that reaches the stem and moves in the flow of perspiration without losses, will accumulate in sites of increased perspiration. This is normal in mature leaves.

With the purpose of analyzing the behavior of the bio-concentration of chlorpyrifos in the tissues of the root and foliage grass *C. clandestinus*, a non-parametric Kruskal-Wallis one-way variance analysis was made. Since the p-value of the F test of analysis of variance is less than 0.05, it is concluded that there is statistically significant difference between the concentrations of chlorpyrifos in the two arrays, root and foliage, with a level of confidence of 95 (Figure 6).

The percentage of chlorpyrifos accumulation in foliage with respect to accumulation in the root is the 4.23 to the maximum concentration of the pesticide treatment and greater exposure time.

CONCLUSIONS

The chlorpyrifos samples by grass *C. clandestinus* is the first stage for bioaccumulation in the food chain, but it is important to understand first the effects of mechanisms of metabolism in plant tissue, since these chemicals can produce secondary metabolites which in some cases

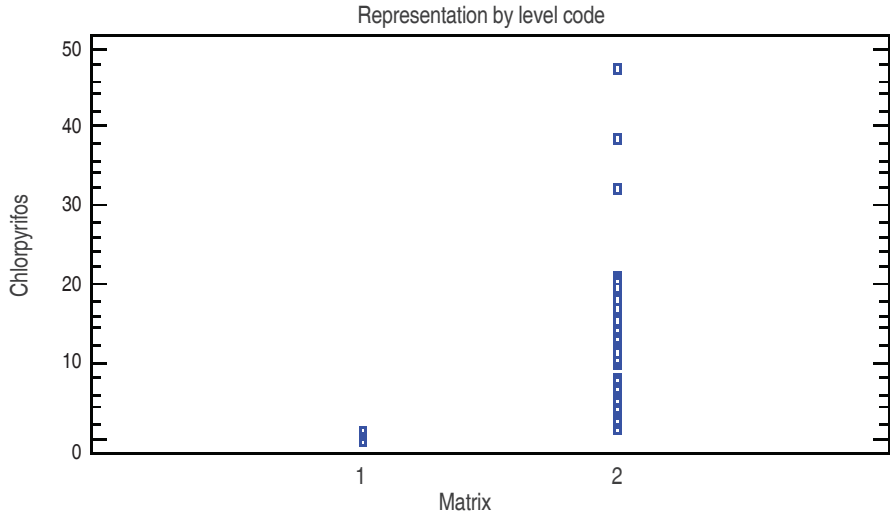

Figure 6. Difference in the concentrations of chlorpyrifos in matrices root and foliage of *C. clandestinus.*

can be more or less dangerous than the original; and the mechanisms of elimination, having clarity in this aspect, it is possible to program the time of natural degradation of chlorpyrifos in the grass required for these processes and the age for the consumption of the grass by cattle.

As conclusion of this investigation, it is highlighted the fact that the chlorpyrifos is a systemic product, which is transported through the vascular system of the grass *C. clandestinus* and is bio-concentrated in the tissues of the plant, with greater emphasis on the root. Bio-concentration in *C. clandestinus* hydroponically cultivated was 39.4 µg g^{-1} to the root and 1.1 µg g^{-1} for foliage. Therefore, the use of this insecticide on livestock systems of high tropical represents a risk for the cattle and other members of the dairy food chain and therefore, the development of agro-ecological farming systems in which chemicals are not used for pest control is indispensable, and instead, an agroecological management was developed for them.

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Association of TLR6 gene polymorphisms with zootechnical parameters in Holstein cattle in northern Antioquia

Asociación de polimorfismos del gen TLR6 con parámetros zootécnicos en ganado Holstein del norte de Antioquia

doi: 10.15446/rfna.v70n2.60766

Juan Pablo Arismendy Morales[1]*, Albeiro López Herrera[1] and José Julián Echeverry Zuluaga[1]

ABSTRACT

Specialized dairy systems are one of the most important lines in the agricultural sector in Colombia and the world. Currently there are strategies, both of environmental management and animal breeding, seeking to optimize the production. TLR6 gene, as many others related to immunity, is proving to be a useful candidate for programs of Marker Assisted Selection (MAS). Four hundred thirty two Holstein cows from 4 municipalities of northern Antioquia were genotyped using PCR-RFLP for C1859A and A1980G polymorphism of TLR6 gene. Allelic frequencies of 57.87% were found for C allele and 42.13% for A allele of C1859A, and 57.99% for A allele and 42.01% for G allele of A1980G. Genotypic frequencies of C1859A were 84.26% and 15.74% for CA and CC, respectively, and no AA genotype for this polymorphism was found; phenotypic frequencies of A1980G were 25.93%, 64.12% and 9.95% for AA, GA and GG, respectively. Mixed models for repeated measures with random effect of the animal were used to determine the association between genotypes and milk yield, percentages of protein and fat, services per conception, calving interval, and SCS. Both polymorphisms were only significant (P<0.01) on the SCS feature, being C allele of C1859A and A allele of A1980G the most favorable. TLR6 gene showed an effect on quality milk trait SCS, as expected for its immunological role in the response against bacteria, and can be postulated as molecular marker for selection against infectious diseases of importance in animal production, such as mastitis.

Keywords:
Allele
Cattle
Genotype
Immunity
PCR
RFLP

RESUMEN

Los sistemas de lechería especializada son uno de los renglones más importantes en el sector agropecuario de Colombia y del mundo. Actualmente existen estrategias, tanto de manejo ambiental como de mejoramiento animal, que buscan optimizar la producción. El gen TLR6, como muchos relacionados con la inmunidad, está demostrando ser un candidato útil para los programas de selección asistida por marcadores moleculares (MAS). Mediante PCR-RFLP, se genotipificaron 432 vacas Holstein de 4 municipios del Norte de Antioquia para los polimorfismos C1859A y A1980G del gen TLR6. Se encontraron frecuencias alélicas de 57,87% para el alelo C y 42,13% para el alelo A de C1859A, y 57,99% para el alelo A y 42,01% para el alelo G de A1980G. Las frecuencias genotípicas de C1859A fueron 84,26% y 15,74% para CA y CC, respectivamente, y no se halló el genotipo AA para este polimorfismo; las frecuencias fenotípicas de A1980G fueron 25,93%, 64,12% y 9,95% para AA, GA y GG, respectivamente. Se utilizaron modelos mixtos de repeticiones con efecto aleatorio del animal para determinar asociación entre los genotipos y las características zootécnicas producción de leche, porcentajes de proteína y grasa, servicios por concepción, IEP y SCS. Ambos polimorfismos solo tuvieron efecto significativo (P<0,01) sobre la característica SCS, siendo el alelo C de C1859A y el alelo A de A1980G los más favorables. El gen TLR6, mostró un efecto sobre el parámetro SCS de calidad de leche, como se espera por su role inmunológico en la respuesta contra bacterias, y puede ser postulado como marcador molecular para la selección en contra de enfermedades infecciosas de importancia en producción animal, como la mastitis.

Palabras claves:
Alelo
Bovinos
Genotipo
Inmunidad
PCR
RFLP

[1] Facultad de Ciencias Agrarias. Universidad Nacional de Colombia. AA 1779. Medellín, Colombia.
* Corresponding author <jparismendym@unal.edu.co>

Received: September 19, 2016; Accepted: February 16, 2017
Rev.Fac.Nac.Agron. 70(2): 8203-8211. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

The dairy sector is one of the leading in agricultural production in the world, being milk the main livestock product in most countries where it is produced (Hemme *et al*., 2012; Knips, 2005). In Colombia, this sector covers 6.4% of dairy herds, producing up to 6716 million liters in 2014 (FEDEGAN, 2014, 2015). In specialized dairy, predominantly in the department of Antioquia, the most common breed is Holstein, which extensive information has been collected in recent years due to record management (Jaramillo and Areiza, 2012).

To optimize production and reproduction, especially in specialized dairy, modern techniques and technologies have been adopted, particularly regarding the selection of the animals and their traits. One of these techniques is Marker-Assisted Selection (MAS), which uses gene variations that confer advantage as a selection criterion (Singh *et al*., 2014). One of the candidate genes to be used as a molecular marker is TLR6, which is a receptor of antigen-presenting cells that can form complexes with TLR2 (Takeda and Akira, 2005), and together may recognize different bacterial antigens, especially zymosan, lipopeptides and lipopolysaccharide (Kang *et al.,* 2011). TLR receptors, including TLR6, have been linked with reproduction and immunological traits (Kannaki *et al*., 2011) as resistance to bacterial diseases like tuberculosis (Sun *et al*., 2012; Song *et al*., 2014) or mastitis (Wang *et al*., 2007; Russell *et al*., 2011) and quality milk traits like SCS (Sharma *et al*., 2006; Chu *et al*., 2009; Zhang *et al*., 2009; Beecher *et al*., 2010).

Every day, the demand for dairy products in our country grows and zootechnical ways for supply it are needed. Due to TLR's role in immune response and health of the animal, and due to only Ramírez *et al*. (2014) have studied TLR receptors and their effect on cattle traits in Colombia, it is important to know, first, the circulating TLR6 polymorphisms genotypes in our cattle populations, and second, if there is a significant effect of the gene on zootechnical traits; all of this for a better designing of breeding and genetic improvement programs in dairy herds.

The aim of this study was to associate C1859A and A1980G polymorphisms of TLR6 gene to productive and reproductive parameters of Holstein cattle in northern Antioquia, to determine favorable alleles for these parameters.

MATERIALS AND METHODS

Place and experimental units

Blood samples from 432 Holstein cows of municipalities of the northern subregion of Antioquia (Colombia) Belmira, San Pedro de los Milagros, and Entrerríos, and from the small town of San Felix, municipality of Bello, were taken. These cows belong to the project "Genetic Evaluation and Dairy Control Program" from the BIOGEM group of the Universidad Nacional de Colombia, Medellín Campus, and Colanta LTDA.

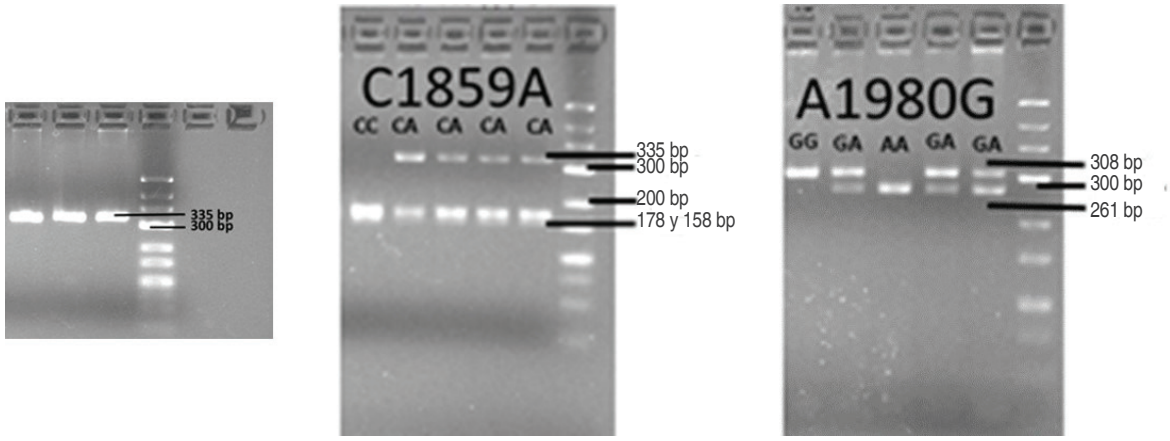
Blood samples were taken by puncture in the middle coccygeal vein and were collected in BD Vacutainer tubes with purple system (EDTA as anticoagulant). They were kept under refrigeration until processing (extraction of DNA), in the Laboratory of Basic Animal Science at the Universidad Nacional de Colombia, Medellín Campus.

DNA extraction and genotyping by PCR-RFLP

DNA extraction was carried out by salting out procedure (Miller *et al*., 1988). For amplification by PCR and genotyping by RFLP for C1859A and A1980G polymorphisms, primers and protocols described by Song *et al.* (2014), which amplify from position 1689 to 2023 of exon 3 of TLR6 gene, according to the sequence NM_001001159 reported in GenBank, were used. Primers used were F: 5'-GGTATCTTTAGCAGCCTTTCCATAC-3' and R: 5'-GTCACAGCAACAGCCAGCA-3' and PCR protocol was done in a total volume of 25 µL containing 0,4 mM of each dNTP, 1 µM of each primer, 2.5 µL of 10X buffer, 2.5 µL of 25 mM $MgCl_2$, ultrapure water, 1 unit of Taq DNA polymerase and from 100 to 200 ng of genomic bovine DNA. Amplification was done with the following program of thermocycling: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, alignment at 65 °C for 30 s, extension at 72 °C for 30 s, followed by a final extension at 72 °C for 7 min. The expected bands are 335 bp.

Digestion with BstXI enzymes (for C1859A polymorphism) and BclI (for A1980G polymorphism) was performed in a final volume of 10 µL, including 3 µL of the PCR product, 5 units of restriction enzyme, 1 µL of the corresponding buffer and ultrapure water. Reactions were incubated at 37 °C for 16 hours and visualized on 3% agarose gels stained with EZ-Vision. The bands obtained by digestion with BstXI were of 335 bp for AA genotype, 335 bp/178

bp/158 bp for CA genotype and 178 bp/157 bp for CC genotype. The bands obtained by digestion with BclI are 308 bp/27 bp for GG genotype, 308 bp/261 bp/47 bp/27 bp for GA genotype and 261 bp/47 bp/27 bp for AA genotype. Bands of 47 and 27 bp are not displayed on the gel due to its size and staining resolution (Figure 1).



The last well of each electrophoretic run image corresponds to the molecular weight marker.

Figure 1. PCR and RFLP banding patterns.

Frequencies calculation and Hardy – Weinberg equilibrium

Frequencies were calculated as the proportion between alleles, genotypes or haplotypes of each type and the total of alleles, genotypes or haplotypes in the population, as Hartl described it in 2007. Hardy – Weinberg equilibrium state was calculated with allelic frequencies observed and expected in software GenAlEx 6.503 (Peakall and Smouse, 2012).

Evaluated productive and reproductive parameters

All information of the animals and of all animal lactations were compiled by the "Genetic Evaluation Program and Dairy Control" for 5 years. The parameters evaluated were total milk yield (TMY) in Kg, percentages of protein and fat in milk per lactation (PP and FP), amounts of protein and fat in milk per lactation (PA and FA) in Kg, calculated with each percentage and the total milk yield, calving interval (CI), services per conception (SxC), and somatic cell score (SCS), calculated by logarithmic transformation of somatic cell count in milk (SCC) with the formula:

$$SCS = Log2(SCC/100) + 3$$

Data were adjusted by eliminating extreme values that could alter the performance of the model; lactations under 250 days and over 450 days, TMY under 2500 kg and over 12000 kg and SxC equal to 0 were removed.

Association of polymorphisms with productive and reproductive parameters

Descriptive analysis of the evaluated parameters was made and frequency tables were used to characterize averages of the parameters according to genotype. To make the association, a mixed model with random effect of the animal and Tukey's range test was used. The models for each trait were evaluated by the Bayesian Information Criterion (BIC), which value indicates the quality of the model; while the value is lower, the model fits better. A linear regression was used to assess the effect of the substitution of each allele, and averages were evaluated. All calculations were made with SAS® software version 9.2 for Windows (SAS Institute Inc, Cary NC, USA).

The mixed model was:

$$Y_{ijklmnop} = \mu + N_i + (H_j * A_k * M_l) + G_m + L_n + P_o + E_{ijklmnop} \quad (1)$$

Being:

$Y_{ijklmnop}$: Productive or reproductive trait

μ: Population average of productive or reproductive trait

N_i: Effect of number of calvings

Contemporary group:
H_j: Effect of herd
A_k: Effect of calving year
M_l: Effect of calving month
G_m: Fixed effect of the gene (genotype)
L_n: Effect of lactation duration
P_o: Effect of the total milk yield per lactation
$E_{ijklmnop}$: Random experimental error
For TMY trait, P_o variable was not used

The linear regression model was:

$$Y_{ijk} = \beta_o + \beta_i L_i + \beta_j G_j + \beta_k P_k \qquad (2)$$

Being:
Y_{ijk}: Productive or reproductive trait
β_0: Intersection or constant
β_i, β_j, β_k: Parameters of each variable
L_i: Effect of lactation duration
G_j: Fixed effect of the alleles
P_k: Effect of the total milk yield per lactation

RESULTS AND DISCUSSION

C1859A and A1980G polymorphisms of TLR6 gene of 432 animals from the municipalities of Bello, Belmira, Entrerríos and San Pedro de los Milagros were amplified. The first polymorphism generates no amino acid change and the second causes a change from isoleucine to valine, which, although is also an apolar and hydrophobic amino acid, has lower molecular weight. The three genotypes of A1980G polymorphism were found, but for C1859A polymorphism only CA and CC genotypes were found in the analyzed population (Table 1). The most frequent alleles were C for C1859A polymorphism and A for A1980G polymorphism; the most frequent genotypes were heterozygous ones in both polymorphisms and, in the case of A1980G, GA is followed by AA genotype. C1859A/A1980G most common haplotype was the CA/GA, followed by CC/AA, CA/AA and CA/GG. By municipality CA/GA remains the most common haplotype, followed by CC/AA, except in Bello, where it is followed by CA/GG; in Belmira, CA/AA and CA/GG haplotypes are equally represented, as are CC/AA and CA/AA haplotypes in Entrerríos. AA genotype of A1980G polymorphism was the only one found with the two genotypes of C1859A polymorphism; the other two were always found with genotype CA of C1859A.

Genotypic and allelic frequencies found are similar to the only two papers reported for these polymorphisms. Song *et al.*

Table 1. Allele, genotype and haplotype frequencies by municipality.

	Polymorphism		Bello	Belmira	Entrerrios	San Pedro	Total
C1859A	ALLELES	A	45	44.44	41.77	39.78	42.13
		C	55	55.54	58.23	60.22	57.87
	GENOTYPES	CA	90	88.89	83.54	79.56	84.26
		CC	10	11.11	16.46	20.44	15.74
		AA	0	0	0	0	0
A1980G	ALLELES	A	51.36	55.54	61.39	59.85	57.99
		G	48.64	44.44	38.61	40.15	42.01
	GENOTYPES	AA	15.45	14.81	32.91	28.47	25.93
		GA	71.82	81.48	56.96	62.77	64.12
		GG	12.73	3.7	10.13	8.76	9.95
HAPLOTYPES C1859A/A1980G		CA/AA	5.45	3.7	16.45	8.03	10.18
		CA/GA	71.82	81.48	56.96	62.77	64.12
		CA/GG	12.73	3.7	10.13	8.76	9.95
		CC/AA	10	11.11	1645	20.44	15.74
		CC/GA	0	0	0	0	0
		CC/GG	0	0	0	0	0

(2014) reported frequencies in Holstein cattle in China above 70% for CA genotype of C1859A polymorphism, followed by CC and AA, which was below 10%, which shows its low frequency in other populations of Holstein cattle and allows to suggest that this allele may be disappearing due to the selection processes that Holstein breed has been subjected to. For A1980G polymorphism, the most common genotype was GA, having a frequency of almost 60%. Chu *et al*. (2009) did not find AA genotype either and found allelic and genotypic frequencies in Holstein cattle from China very similar to those found in this work; reporting allele frequencies of 58.9% for C and 41.1% for A; and genotype frequencies of 17.8% for CC and 82.2% for CA. For A1980G polymorphism, allele frequencies of 54.3% for A and 45.7% for G; and genotype frequencies of 26% for AA genotype, 56.7% for GA and 17.3% for GG were found (Chu *et al*., 2009).

In Table 2 are shown the X^2 and P values of Hardy – Weinberg equilibrium state analysis. All polymorphisms in all municipalities are in disequilibrium state, except A1980G polymorphism in Belmira. As said above, the artificial selection processes the Holstein cattle suffer may be causing the frequencies of some alleles of TLR6 polymorphisms are reducing.

In Table 3, the mean and CV of the analyzed traits are shown.

Table 2. Hardy – Weinberg Equilibrium analysis.

Municipality	Locus	Degree of freedom	X^2	*P* value
Bello	C1859A	1	50.77	<0.01**
Bello	A1980G	1	12.00	<0.01**
Belmira	C1859A	1	7.96	<0.01**
Belmira	A1980G	1	1.98	0.16
San Pedro de los Milagros	C1859A	1	76.08	<0.01**
San Pedro de los Milagros	A1980G	1	10.17	<0.01**
Entrerríos	C1859A	1	97.44	<0.01**
Entrerríos	A1980G	1	27.59	<0.01**

Values with ** are very significant (*P*<0.01)

Table 3. Mean of the evaluated trait.

Trait	Mean	Standard deviation	CV
Lactation Period (days)	333.43	48.99	14.69
Total Milk Yield (kg)	5643.20	1746.28	30.94
Protein Porcentage	2.98	0.23	7.80
Protein Amount (kg)	155.53	37.97	24.41
Fat Porcentage	3.77	0.46	12.27
Fat Amount (kg)	197.59	55.32	28.00
Services per Conception	1.78	1.20	67.28
Calving Interval (days)	444.76	159.28	35.81
Somatic Cell Score	4.34	1.32	30.49

The average milk yield found in this work (5643.2 kg) is higher than that recorded by Quijano *et al*. (2011), 5140 kg per lactation; protein (2.9%) and fat percentage (3.77%) of this study are similar to those reported by this author (3.07% and 3.8% respectively), but the CI (445 days) was greater than the one they found (417

days). The Holstein Association of Colombia recorded a higher average yield of (6237 kg) and SxC (2.57). Jaramillo *et al*. (2012), reported for the so called Region 1, which covers departments known for its dairy orientation including Antioquia, protein and fat percentages of 3.07 and 3.63 respectively for 2012; nationwide, they reported 3.1% and 3.66% respectively. The SCS found in this study was considerably higher than the 3.06 reported by Quijano *et al*. (2011), probably due to the smaller sample number and the smaller number of analyzed regions in this work. Gaviria *et al*. (2014) also reported SxC of 1.66 in different municipalities of Antioquia, being more favorable the results of this study. Also they recorded in different municipalities of the northern region of Antioquia an adjusted milk yield to 305 days of 5588 kg, protein and fat percentages of 3.06 and 3.89 respectively, a SCS of 4.19, a CI of 414 days and a SxC of 1.67; these results show a better trend than those found in this work, with higher yields and lower CI and SCS (Gaviria *et al*., 2014, 2015).

The association analysis showed that none of the polymorphisms had a significant effect on the evaluated trait, except for SCS, in which the effects were significant (P<0.01), including the effect of the haplotype (Tables 4 and 5). Since from C1859A polymorphism only CA and CC

Table 4. Trait means of C1859A genotype.

Trait	P value in the model	C1859a Polymorphism			
		CA		CC	
		Average	CV	Average	CV
TMY	0.59	5482.27	31.30	5525.01	32.34
PP	0.82	3.00	8.31	2.96	7.59
PA	0.56	151.90	24.86	149.68	24.37
FP	0.99	3.75	12.56	3.70	14.17
FA	0.97	190.62	28.27	187.16	27.65
SxC	0.62	1.57	68.68	1.62	66.77
CI	0.53	84.01	37.49	452.22	34.91
SCS	<0.01 **	4.47 [a]	30.99	4.06 [b]	31.87

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (P<0.01). Values with different superscripts indicate significant statistical difference

Table 5. Trait means of A1980G genotype.

Trait	P value in the model	A1980G POLYMORPHISM					
		AA		GA		GG	
		Average	CV	Average	CV	Average	CV
TMY	0.99	5444.52	30.64	5534.17	31.49	5326.05	33.39
PP	0.76	3.00	8.03	3.00	8.34	2.97	7.96
PA	0.79	150.17	22.67	153.76	25.69	141.29	22.63
FP	0.96	3.73	14.14	3.75	12.59	3.74	10.49
FA	0.99	186.69	26.05	193.27	29.15	178.98	25.71
SxC	0.92	1.57	69.67	1.6	69.36	1.54	68.45
CI	0.32	456.37	32.58	454.47	40.15	450.21	27.74
SCS	<0.01 **	4.19 [a]	33.53	4.52 [b]	30.50	4.24 [ab]	28.82

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (P<0.01). Values with different superscripts indicate significant statistical difference

genotypes were found, were these genotypes the ones that had significant differences (*P*=0.0042); in the case of A1980G polymorphism, which both homozygous genotypes were found, there was a significant difference between GA and AA genotypes (*P*=0.0143). The difference of SCS between C1859A/A1980G haplotypes, with *P*=0.0087, was among CA/GA and CC/AA (Table 6). Both statistical association and the comparison of averages between genotypes and haplotypes indicate a favorable effect of C allele of C1859A and A allele of A1980G on the SCS feature.

Table 6. Trait means of C1859A/A1980G haplotype

Trait	P value in the model	Haplotypes C1859A/A1980G							
		CA/AA		CA/GA		CA/GG		CC/AA	
		Average	CV	Average	CV	Average	CV	Average	CV
TMY	0.98	5324.92	27.78	5.534.17	31.49	5326.05	33.39	5525.01	32.34
PP	0.84	3.05	8.33	3.00	8.34	2.97	7.96	2.96	7.59
PA	0.72	150.85	20.29	153.76	25.69	141.29	22.63	149.68	24.37
PG	0.95	3.77	14.13	3.75	12.59	3.74	10.49	3.70	14.17
FA	0.99	186.05	23.82	193.27	29.15	178.98	25.71	187.16	27.65
SxC	0.93	1.46	64.37	1.6	69.36	1.54	68.45	1.62	66.77
CI	0.45	462.01	29.46	454.47	40.15	450.21	27.74	452.22	34.91
SCS	<0.01**	4.38^{ab}	35.23	4.52^{a}	30.50	4.24^{ab}	28.82	4.06^{b}	31.87

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS
Values with ** are very significant (*P*<0.01). Values with different superscripts indicate significant statistical difference

In the work of Chu *et al*. (2009) it was found that A allele of A1980G polymorphism confers resistance to mastitis, giving SCS values significantly lower than the G allele, but found no association between C1859A polymorphism and mastitis. Chu *et al*. (2009) also associated other polymorphisms of TLR6, T853A and G855A, with mastitis, being T and G alleles, respectively, which confer resistance. In addition, Song *et al*. (2014) related both polymorphisms with resistance to tuberculosis, being alleles C of C1859A and G of A1980G favorable, demonstrating that these polymorphisms may be useful selection molecular markers against bacterial diseases.

In both polymorphisms, homozygous genotypes were more favorable than heterozygous genotypes, that is, they had lower SCS values. Although genetic variability (heterozygosity) is usually desirable in immune response related genes (Penn *et al*., 2002) and usually heterozygous genotypes of these genes confer some advantage, for the so - called “hybrid vigour” or “heterosis”, for these polymorphisms, homozygous genotypes are the ones that confer advantage in the SCS trait. Since SCS is usually related to clinical and subclinical mastitis and can serve as an indirect indicator of this type of disorders, a possible explanatory hypothesis offered in this work to the apparent advantage conferred by homozygosity in the studied population is based on the antibacterial function of TLR6.

It is known that several bacterial pathogens are related to mastitis, but the main reported in Colombia are *Staphylococcus aureus, Streptococcus agalactiae,* and *Streptococcus dysgalactiae*, and its prevalence is always the highest but it can be sectioned and depends on the region (Calderón and Rodriguez, 2008; Ramírez *et al*., 2011; Rangel *et al*., 2011; Trujillo *et al*., 2011). Since this 3 species of bacteria are the main cause of mastitis in Colombia, and bacteria of this same genus are highly related, it may be the case that a single allele of immunity is favorable or effective against all of them and genetic variability seems not so necessary. Similarly, as homozygous individuals for this favorable allele can produce more protein product of the same type, and if this product is the most effective then are these individuals who have the advantage over the heterozygous individuals.

A contrasting example of what was found in this work is what Chu *et al*. reported in 2009, who found that the

more favorable allele of A1980G was G, suggesting that the effect of this allele is more effective against pathogenic microbiota own of Chinese Holstein cattle, which is probably different from the pathogenic microbiota in Colombia; on the other hand, they found significant differences among all genotypes, being GG the better, followed by GA and this followed by AA, which does not match with the findings of the present study. More studies are needed then, where the number of herds, number of animals and data per animal are higher and allow more reliability and more concrete results that match or refute these studies.

The analysis of allelic substitution to establish the trait changes with each change of allele only yielded significant effect on the SCS (Table 7). For C1859A polymorphism changing each C for A is unfavorable for TMY, SXC, CI and SCS, while changing each A for G in A1980G polymorphism is unfavorable for PP, PA and SCS. The change from CC to AA in the C1859A polymorphism can generate an increase up to 0.74 and the change from AA to GG in A1980G polymorphism can generate an increase up to 0.22 in SCS.

Table 7. Changes in traits with each allelic substitution.

TRAIT	POLYMORPHISMS	
	C1859A C>A	A1980G A>G
TMY	-23.21	23.47
PP	0.044	-0.001
PA	2.42	-0.56
FP	0.04	0.008
FA	3.2	0.99
SxC	0.04	-0.001
CI	2.39	-3.59
SCS*	0.37	0.11

Total milk yield in kg: TMY, percentages of protein and fat in milk per lactation: PP and FP, amounts of protein and fat in milk per lactation in kg: PA and FA, calving interval: CI, services per conception: SxC, somatic cell score: SCS. Values with * are significant (P<0.05)

CONCLUSIONS

Allelic and genotypic frequencies of C1859A and A1980G polymorphisms of TLR6 gene for 432 Holstein cows from northern municipalities of Antioquia were determined. The most common alleles were C for the first polymorphism and A for the second, being heterozygous ones the most common genotypes; although analyzing allelic frequencies, not allele fixation is shown, it is possible that A allele of C1859A may be suffering a process of negative selection in improved Holstein cattle, since no AA genotype was found in the population, which is concordant with reports made by other authors. The only significant effect of polymorphisms was found on the SCS trait, expected result because this gene is involved in the immune response against bacterial pathogens; C allele of C1859A and A allele of A1980G were the most favorable alleles. The results were not completely conclusive because the heterozygous genotypes were the worst performers, contrary to expectations, especially in immune-related genes. Probably homozygosity is favorable due to the relatively narrow spectrum of main mastitis pathogens. More studies that better characterize the genotype of the population of Holstein cattle in dairy municipalities of Antioquia are needed and for they continue to design better selection strategies, especially when the goal is to improve SCS without altering or changing other traits.

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Revista Facultad Nacional de Agronomía

UNIVERSIDAD NACIONAL DE COLOMBIA

Artisanal mining and the use of plant diversity

La Minería Artesanal y el uso de la diversidad vegetal

doi: 10.15446/rfna.v70n2.64525

Fabio García-Cossio[1], Heiler Cossio-Mosquera[1], Bertha Conto García[2]*, Vianney Sarria Palacios[2] and Luis Emilio Conto García[3]

ABSTRACT

This essay presents the variety of vegetation which is utilized in traditional mining activities in the municipalities of Cértegui (Subdistricts of: Cértegui cabecera, La Toma y Recta Larga) and the Panamerican Union (Subdistricts of Animas, Agua Clara and Quiadó). The data is a product of an ethnographic investigation which selected 57 active traditional miners of the local population in the project's area of influence to demonstrate a sample of the "Application of techniques and practices for cleaner production in gold and platinum mining in the department of Chocó". These miners aided in recognizing, collecting, photographing, and identifying 78 species of vegetation used in activities associated with traditional mining such as: separation of metals (separating gold from jagua, settling the greasy gold and blackening the troughs), creating traditional tools (troughs, sifters, mining tools to store save and weigh gold) to work in different types of traditional mining ('guaches' or pits, mazamorreo or barequeo (gold-panning), hoyadero (underground mining), zambullidero (underwater mining), canalon (sluice box), agua corrida (streaming water), cuelgas (channels) and arrimadero), and confirming the affectivity by performing demonstrations of the separation of gold from jagua with mucilaginous strata of vegetation. Examples of this vegetation include: snakewood (*Cecropia peltata*), rhombus-leaved sida (*Sida rhombifolia*), Common Broom (*Pavonia fruticosa*), Shoeblackplant *(Hibiscus rosa-sinensis*), Balsa tree (*Ochroma pyramidale*) and Guácimo (*Apeiba tibourbou*).

Key words:
Mining tools
Types of mining
Species of vegetation
Chocó
Colombia

RESUMEN

Se presenta el inventario de las especies vegetales utilizadas en las actividades de la minería artesanal en los municipios de Cértegui (Cértegui cabecera, La Toma y Recta Larga) y Unión Panamericana (Ánimas, Agua Clara y Quiadó), los datos fueron producto de una investigación etnográfica en la cual se seleccionaron 57 mineros artesanales activos como muestra poblacional de los sitios beneficiarios del proyecto "Aplicación de técnicas y prácticas de producción más limpia en la minería auro-platinífera del departamento del Chocó" quienes permitieron reconocer, colectar, fotografiar e identificar 78 especies vegetales utilizadas en las actividades de minería artesanal como son: separación de metales (separar oro de jagua, asentar el oro grasoso y negrear las bateas), elaboración de herramientas artesanales (bateas, cachos, cabos de herramientas, para guardar y pesar oro), elaboración de equipos artesanales (matracas, malacates y elevadores) y para la elaborar los tipos de minería artesanal (guaches o socavón, mazamorreo, barequeo, hoyadero, zambuyidero, canalón, agua corrida, cuelgas, arrimadero) y comprobar su efectividad con la realización de cinco ensayos de separación de oro de la jagua con estratos mucilaginosos de especies vegetales como: Guarumo o Yarumo(*Cecropia peltata*), Escobababosa (*Sida rhombifolia*), Escoba negra (*Pavonia fruticosa*), Resucito *(Hibiscus rosa-sinensis*), Balso (*Ochroma pyramidale*) and Guácimo (*Apeiba tibourbou*).

Palabras claves:
Utensilios mineros
Tipos de minería
Especies vegetales
Chocó
Colombia

[1] Corporación Agroambiental del Trópico - CATRÓPICO. Calle 22 No. 22-14. Quibdó, Chocó.
[2] Universidad Tecnológica del Chocó Diego Luis Córdoba. AA 292. Quibdó, Chocó.
[3] Corporación Autónoma Regional para el Desarrollo Sostenible del Chocó - CODECHOCO. Carrera 1 No. 22-96. Quibdó, Chocó.
* Corresponding author: <beconto@yahoo.com>

Received: Marzo 29, 2016; Accepted: Febrero 21, 2017
Rev.Fac.Nac.Agron. 70(1): 8213-8223. 2017 ISSN 0304-2847 / e-ISSN 2248-7026

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The Spanish conquistadors occupied the territory which is currently Colombia and they stripped the indigenous people of their gold at the beginning of the XVI century. When this period ended, many of them went to rivers and ravines which the natives had shown them to sift through the sand to obtain precious metals from their natural sources. This is how mining was born in our country (Poveda, 2002). Mining in Colombia has been a common activity since the colonial period despite not having been an economic leader or representative of the country until the beginning of this century in which a global boom in the demand for minerals and an increase in the price has awakened the interest of foreign investors and the central government (Ronderos, 2011).

The rivers in Colombia, which are born from three mountain ranges, were loaded with gold and silver in grains dispersed through the river sands in the deep silt (Poveda, 2002). The "trough" or "cuna" was sufficient for the Spaniard or Mestizo with enough physical fortitude, ambition, and tenacity to dedicate years to this work and eventually become rich (Poveda, 2002). During more than three centuries, neogranadine mining worked in this simple and primitive manner. When the indigenous people workforce had been exhausted due to mortality (Poveda, 2002) and their resistance to forced labor which they always opposed, the Spaniards saw the necessity to import slave labor for mining activities (West, 2000). Spain authorized the bringing of black Africans to Cartagena, where they were sold to their new masters who brought them to their respective mining regions where they were required: Antioquia, Chocó, Alto Cauca, and the Valley of Patia. During the first years of the XX century, precious metals continued to be the only products of national mining (Poveda, 2002).

The Colombian Pacific region had historically been practicing a traditional mining activity which is called "barqueo". For this activity, large machines were not used. On the contrary, human labor was used to extract minerals in a traditional way, obviously in small quantities (González, 2013). This is the type of mining which was performed by Afro-Colombian commoners (Vergara, 2007). Under the vision imposed by the members of the network for responsible mining (RESPOMIN) and the Alliance for Responsible Mining (ARM), Traditional Small Scale Mining (MAPE) was conceived as: A formalized activity which was organized, profitable, used efficient technologies and was socially and environmentally responsible. It has increasingly developed as a framework for governance, legality, participation and respect for diversity, and has increased its contribution to countries by generating dignified employment, local development, fight against poverty and social peace. It is stimulated by an increasing demand for sustainable minerals and jewelry for consumers (RESPOMIN, 2007).

At the Cocoana region, the exploitation of gold has been seen as an activity with an ancestral character, however, the indiscriminate practice has become a strategy of territorial colonization which deteriorates the environment and causes high levels of poverty and social inequality (Álvarez, 2013).

Mining since the XVI century has maintained the traditional systems of production which dominated the slavery period. About 30 municipalities are entirely dedicated to gold and platinum material extraction. This highlights that traditional mining has historically been sustained in environmentally friendly practices, which perhaps are done with traditional tools which support their own traditional technologies or ethno-technologies which move small volumes of golden nuggets; the environmental impact of which is not wide-spread, significant, or difficult to assimilate with natural processes (Ayala, 2011).

Just as mining has persisted as an economic activity, the forms of organization for this type of activity have also persisted. Understanding this organization is important to understanding the community organization which has surged as a social and political resistance toward the State (Vergara, 2007).

The thousands of informal miners who seek to exploit the gold in the rivers are contaminating the water with heavy metals like mercury and cyanide. Furthermore, amalgams which form with these metals are burned to extract the gold, and the air is also poisoned (Ronderos, 2011). Treating this problem created the project "*Application of techniques and practices of cleaner production and mining of gold and platinum materials in the department of Chocó*" with the purpose of bringing about sustainable production practices to benefit processes related to gold and platinum materials

in the municipalities of Certegui and the Panamerican Union of the department of Chocó. One of its objectives is to implement a process of technological innovation to eliminate the use of mercury and backhoes in small or medium scale mining, and conserve the tradition of using substances of native plants in the excavation of minerals to eliminate the use of mercury.

In the Tumbes-Chocó-Magdalena biodiversity hotspot, between 7000 and 8000 species of vegetation exist, and in the department of Chocó, 3,866 species have been registered (Forero and Gentry, 1989). Many of these have multiple uses for medicine, food, religious magic and traditional crafts according to the register which says that 160 species are used for traditional purposes (Garcia and Restrepo, 1997) which are used by miners. A mucilage substance can also be extracted which ancestrally has most often been used in the process of separating gold from jagua.

This project indirectly benefits the 19,059 inhabitants of the municipalities of Certegui and the Panamerican Union in the department of Chocó and directly benefits the 320 people who work in ten mining entables in this municipality according to a study which has "the design and implementation of the technological process which benefits the development of medium or small scale mining without the use of mercury" as its' second phase.

The lack of knowledge about the experience of afro communities in relation to the use of vegetation species in mining provokes the following questions: What species of vegetation are used in mining in the municipalities of Certegui and the Panamerican Union? Which parts of the vegetation species are employed for the separation of gold and jagua?

To collect this information, the following techniques were applied: polls, interviews, participant observation, classes and documented analysis through strategies like visiting work zones, conversations and consulting bibliographies by which the inventory of vegetation species was obtained and currently recognized by the miners in the area of the study and who are employed in the extraction of gold. Demonstrations in the field and the laboratory of the separation of gold and jaguar using these species were also performed.

MATERIALS AND METHODS

The study was oriented around the Descriptive Method combined with Qualitative Ethnography and Participatory Focus. The first because it is directed at describing the concrete conjunction of identified phenomena, each of which will be analyzed at the moment determined by itself to specify its characteristics (ICFES,1995). The second is about discovering the origin, the reason for the existence of a social fact, or the process of finding the interpretations and relationships. It is not a rigid design, nor does it delineate the steps inflexibly; it is above all adaptable to cultures, situations and contexts, aids the investigator because it is an instrument which may constantly be in revision and is permanently being reconstructed (Watson (1998). This methodology has its' own specific quality. It manifests as much in the data identified with "words" as much as in the treatment characterized by the use of different mathematical and numerical strategies and in the procedures to assure the validity and confidence of the obtained results (Hubert and Marcelo, 1990 cited by Lorenzo, 2011).

From the vision of the participatory method, the enforced regulations on the channels, mechanisms and levels of participation from the citizens and the community were taken into account: Congress of Colombia, Political Constitution, del 4 de julio de 199, Art. 79. Congreso de Colombia. Ley 136 del 2 de junio de 1994, Cap. VIII, Art. 141. Congreso de Colombia Ley 134 del 31 de mayo de 1994, Arts. 98 y 100. Congreso de Colombia, LEY 99 del 22 de diciembre de 1993, Art. 74.

Description of the study area

This project was executed in the municipalities of Certegui and the Panamerican Union, in the department of Chocó Colombia (Figure 1).

Population and Sample

The population is represented by 320 miners of the municipalities of Certegui and the Panamerican Union. To select our sample, the Simple Random Sample method was used because when everything of which the universe is composed is known; everything has an equal chance of being selected for the sample. It was chosen because it is considered to be very practical when the population is not very large and is located in a small area (Instituto Colombiano para el Fomento de la Educación

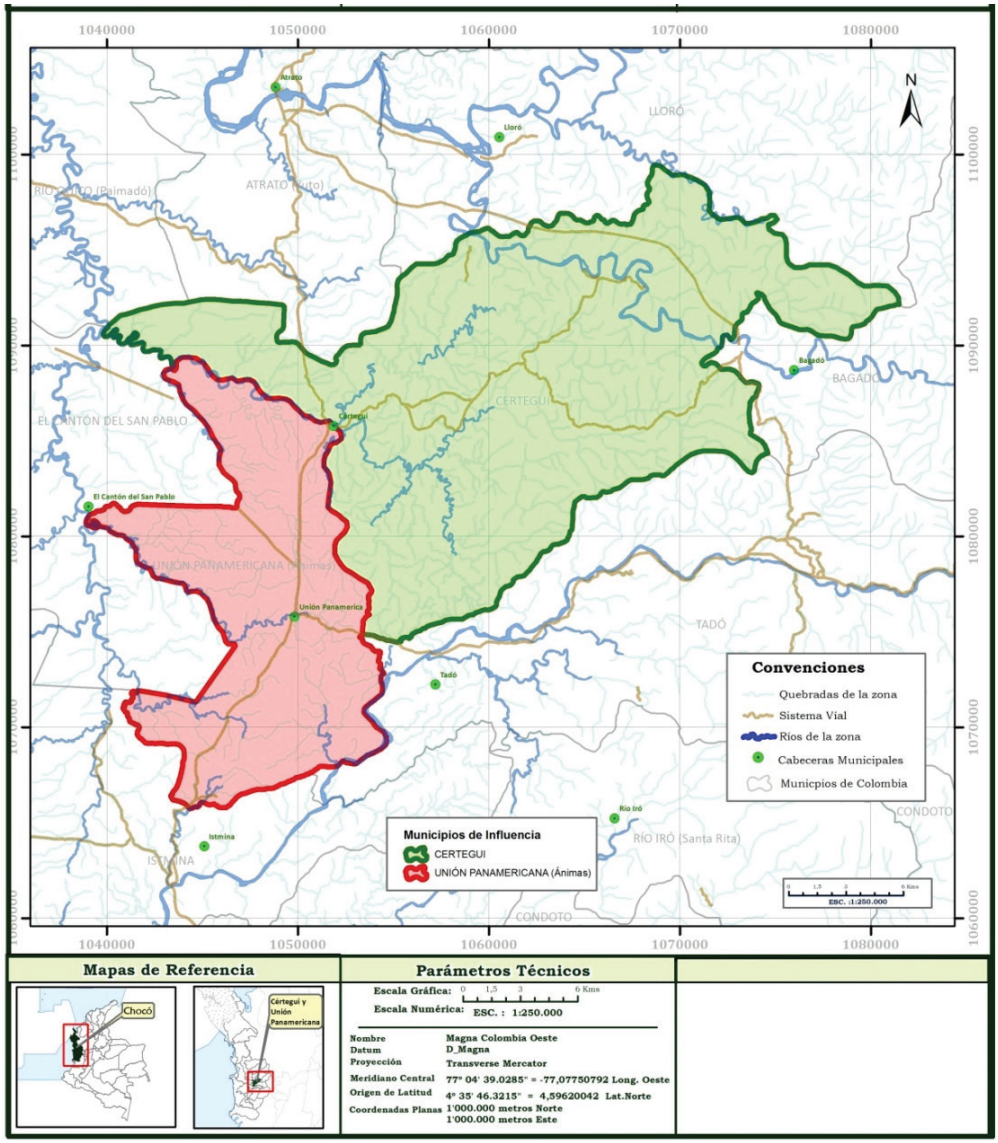

Figure 1. Municipalities of Certegui and the Panamerican Union. Source. Agreement 020 24/12/ 2013 CODECHOCO - UTCH

Superior - ICFES, 1995). The result was that 57 people participated in different identification activities of different vegetation species, information collection, participatory classes and information validation activities.

Strategy for information collection

It begins with the plan for information, applying tactics to awaken the sensibility of the mining community around the project, generating confidence, visiting the study zone, social events and conciliation dialogues. The purpose is to motivate active, experienced miners to make decisions which will promote their free and spontaneous connection to the investigative process.

The primary information was the principal source for summarizing the rest of the information by applying specialized techniques to bring together the information in a qualitative form such as: The Direct Structured Observation, the Structured Ethnographic Interview, the Interview and classes, using index cards with experiential ethnographic questions, observation guides, and instruments which were composed of open and closed questions with the function of numerical weight.

Collection and recognition of vegetation species
With the participating research groups, made up of the mining stakeholders who were part of the population sample, the selected sites were chosen in each municipality. The following sites were chosen: The municipality of Certegui, the municipal head, the township of Toma and the jurisdictional division Recta Larga; In the Pan-American Union, the village of Animas, the village of Quiado and the village of Agua Clara. The vegetation species used to separate the gold from the jagua were collected at these sites. Studies performed in the field were an important means of increasing the number of plant species under study. Binnacles, field journals, cameras and video recorders were used to take morphological data for later identification.

Classes and miner interviews in the selected zones
In the classes for collecting information, groups were formed which created a list of vegetation species used to separate gold from jagua easily, to settle the greasy gold or to ferry as it is known ancestrally, and to blacken the troughs which allows the gold to be seen, especially when it is quite small or minute, individual interviews with some miners were performed which strengthened the lists and the knowledge of the mining extraction in the areas.

Species identification
For comparison with the experiments of the herbarium "CHOCO", from the Technological University of Chocó, which includes websites, databases, scientific collections online, from the database which was constructed with specify, dictionary common names of Colombian plants, from tropical plant guides, neotropical herbarium, and specialized literature were used to identify species with the most specific taxon possible (Missouri Botanical Garden; The Plant List; The Field Museum; Instituto de Ciencias Naturales (ICN) - Universidad Nacional de Colombia).

Experimental field tests: verification of the selected species in each case
With the support and knowledge of the natives, people with greater knowledge and mining tradition, who reported the use of plant species in the separation of gold and jagua and settling gold, gave demonstrations with the species, showing the effectiveness of these for the specific purpose of separating gold and jagua. It consisted of demonstrative practices of separation by rotating the material in the trough, removing the stones until only gold combined with jagua remained and then they continued to rotate as they applied more water and the mucilaginous substance extracted from the vegetation, leaving only gold in the trough. Finally, 5 species with the most tradition were selected and the demonstrations were carried out, with the volume and quantity of material maintained constant to demonstrate the effectiveness of the method.

RESULTS AND DISCUSSION

In the framework of the ethnographic project, six information gathering workshops and 57 interviews with miners of the municipalities of Certegui and the Panamerican Union were carried out, which allowed the registration of 199 samples of individual vegetation samples employed in mining activities, which were distributed into 78 species ; equivalent to 0.97% of the biogeographic species of Chocó, 2.0% of those in the department of Chocó and 48.7% of the artisanally used plants in Chocó, which are classified in 39 botanical families. The most represented families are Malvaceae with 10 species, followed by Fabaceae and Rubiaceae with five each and Melastomataceae, Lauraceae and Annonaceae with 4 species each respectively (Table 1). It is important to emphasize that the higher representation of the Malvaceae and Annonaceae families is due to the fact that all their species have mucilage directly in their structures which were ancestrally used for the separation of gold from jagua. New products were found as a result of the research process because no existing documentation was found with information on the plant species used in traditional mining.

The mining community reports that the vegetation species Cedro Macho (*Tapirira guianensis*) is the most used in the traditional mining troughs. It is a tool which was ancestrally used in barequeo mining.

As a result of the bibliographic review, workshops, surveys and informal talks, four artisanal mining methods are recognized as areas of ethnographic study in which vegetation species are used for accomplishing work or labor, among which we have seen: Separation of the metal (gold), use of artisanal tools, use of artisanal equipment and construction of systems or types of exploitation in traditional mining.

Table 1. Best represented families

Families	No. species	Families	No. Species
Malvaceae	10	Convolvulaceae	1
Fabaceae	5	Euphorbiaceae	1
Rubiaceae	5	Musaceae	1
Lauraceae	4	Caryocariaceae	1
Annonaceae	4	Burseraceae	1
Melastomataceae	4	Lecythidaceae	1
Meliaceae	3	Myrtaceae	1
Apocynaceae	3	Chrysobalanaceae	1
Poaceae	2	Commelinaceae	1
Urticaceae	2	Acantaceae	1
Vochysiaceae	2	Bignoniaceae	1
Olecaceae	2	Ochnaceae	1
Sapotaceae	2	Simaroubaceae	1
Moraceae	2	Gesneriaceae	1
Araceae	2	Elaeocarpaceae	1
Myristicaceae	2	Cyatheaceae	1
Arecaceae	2	Clusiaceae	1
Anacardiaceae	1	Asteraceae	1
Humiriaceae	1	Cucurbitaceae	1
Nictaginaceae	1		

Of the 78 species used in artisanal mining in the areas studied, 48.7% are used in the direct separation of gold from mining lands, 43.5% in the development of manual tools used by traditional miners to facilitate the selection of soil from which the gold is extracted, 21.7% in the production of artisanal equipment and 29.4% in the production of different tools used in traditional mining or in ancestral forms of extracting gold from mining lands (Table 2).

In ancestral mining, the mining communities in Certegui and the Panamerican Union used vegetation species largely in three activities:

Separation of gold from jagua. The process uses the maceration of the vegetation species to generate an emulsion with the obtained mucilage, and proceeds to separate the elements by means of the system of rotation and manual separation in the trough, adding water and the gelatinous emulsion with a consistency which is a product of the experienced miners.

Settling of greasy gold. To settle the greasy gold, or ferry, use the mucilage with a different viscosity, add some to create a muddy solution.

Blacken the troughs. To blacken the troughs, other plant species are used which macerate over it until obtaining the desired effect, which is completed by placing them in the sun. The purpose of blackening is to generate greater visual contrast with the small particles of gold as they are separating. In these processes, 38 plant species were used. The jagua gold separation process was the one with the highest requirement of plant species, with 71.0% being used for this purpose, followed by blackening troughs with 28.94% and settling greasy gold with 10.52%; this input was also new for Chocó because only the work of ASOCASAN (2010)

Table 2. Number of species by artisanal activity.

Activity	No. species	%*
Separation of gold	38	48.7
Development of artisanal tools	34	43.5
Development of artisanal equipment	17	21.7
Different types of traditional mining	23	29.4

* The results are above 100% because some species are used in various different activities.

reports that "we use natural components to cut gold (in the process of separation of gold from the jagua), for which balsa (*Ochroma pyramidale*) A forest species that is neither polluting nor toxic to aquatic life is used (Fermín, 2016)[1].

The species best represented by frequency of community use and registered as being in use by the six communities are: rhombus-leaved sida *(Sida rhombifolia)*, common broom *(Pavonia fruticosa)*, snakewood *(Cecropia peltata)* and blackberry *(Clidemia catroughellata)*. These were used ancestrally in mining to separate gold from jagua which they extracted from the ground and accumulated during the course of the day. The part of the vegetation most used for the separation of gold and jagua are the leaves, corresponding to 23 species followed by the sap with 10 as shown in Table 3. It is important to note that the miners state that "any plant that produces slime (mucilage) can be used in the separation of gold from jagua. You only need to have the experience to take it at the right point" (degree of optimum viscosity).

Table 4 shows the number of plant species used for the production of handcrafted tools, as well as those reported for correction (troughs, tool handles for hoes, shovels, pick axes and machetes, among others. The tool handle is one of the more species registered tools, especially in the correction of Quiadó.

According to Escalante (1971), Córdoba and Rovira (sf), Álvarez (2013), Ayala and García (2011), there are approximately 18 artisanal tools used by the Chocó miners (mud, pick axes, leather, troughs, hoes, spouts, shovels, hoes, machos, wedges, axes, wagons, poles, jagueros, machetes, wooden supports, picks and hollows). Some of these form the so-called tool handles. Our ethnographic work registers five types of tools being tool handles which reports the most species used for this purpose with 28 plant species, followed by troughs with 14 as shown in Table 4. This is why this documentation which reports 34 vegetation species used in the production of artisanal tools is novel.

Table 4 presents the number of vegetation species used by participating communities in these types of traditional mining. The exploitation of the system of Guaches accounts for the largest number of used species as can be observed on the Table 5.

Field trials with the twenty-seven plant species used in the area of study to separate the gold from the jagua with the use of mucilaginous substances demonstrated the effectiveness of this practice which makes it possible to corroborate that this ancestral practice is necessary. It should remain active in miners from generation to generation and thus prevent the use of mercury in this traditional practice.

Table 6 shows the demonstrated exercises with the mining groups while using the most useful and recognized species in the study area. For the practice, a sample weight and constant water volume were defined with the purpose of knowing in the first instance the effectiveness of the vegetation species in this process, thus it was found that the process is more efficient with balsa (*Ochroma pyramidale*) which used only 20 mL of the substance in less than two minutes, followed by common broom (*Pavonia fruticosa*) with 25 mL of the substance.

[1] Artisanal mining. Personal communication. October, 2016.

Table 3. Vegetation species used in the separation of gold by different communities

No	Common Local Name	Scientific Name	Family	Use			Part Used				Location					
											Cértegui			Pan-American Union		
				OJ	N	G	H	T	R	S	C	T	RL	A	AG	Q
1	Baba zaíno	*Cochliostema odoratissimum*	Commelinaceae	X			X					X				
2	Babosa	*Aphelandra* sp	Acanthaceae	X			X				X		X		X	
3	Balso	*Ochroma pyramidale*	Malvaceae	X			X			X			X	X	X	x
4	Batata	*Ipomoea batatas*	Convolvulaceae	X			X							X		
5	Caidita	*Ocotea oblonga*	Lauraceae	X			X	X				X	X			
6	Carbonero	*Licania macrophylla*	Chrysobalanaceae		X		X					X	X			
7	Cargadero trougha	*Annona cargadero*	Annonaceae	X			X			X			X	X		X
8	Cascajero	*Palicourea sp*	Rubiaceae		X		X				X		X			
9	Chocolate	*Theobroma cacao*	Malvaceae	X			X			X				X	X	
10	Chuscal	*Andropogum bicornis*	Poaceae			X	X				X			X		
11	Coronillo	*Bellucia glossularoides*	Melastomataceae		X	X	X				X	X	X		X	X
12	Cuadrado	*Musa balbisiana*	Musaceae		X		X									X
13	Escoba babosa	*Sida rhombifolia*	Verbenaceae	X			X				X	X	X	X	X	X
14	Escoba negra	*Pavonia fruticosa*	Verbenaceae	X			X				X	X	X	X	X	X
15	Guácimo blanco	*Apeiba membranácea*	Tiliaceae	X			X	X		X	X	X	X		X	
16	Guácimo colorado	*Luehea seemnnii*	Tiliaceae	X			X	X		X		X	X	X	X	X
17	Guarumo	*Cecropia peltata*	Urticaceae	X			X				X	X	X	X	X	X
18	Guayacán negro	*Minquartia guianensis*	Olacaceae		x											X
19	Hierba mora	*Clidemia catroughellata*	Melastomataceae	X	X	X	X				X	X	X	X	X	X
20	Jaboncillo	*Isertia alba*	Rubiaceae	X	X								X		X	X
21	Jagua macho	*Genipa* sp	Rubicaeae		X		X									X
22	Malangá	*Xanthosoma sagitifolium*	Araceae	X			X									X
23	Malva	*Malachra alceifolia*	Malvaceae	X			X				X	X	X		X	X
24	Moro grande	*Miconia reducens*	Melastomataceae		X		X				X		X			
25	Palma Zancona	*Socratea exorrhiza*	Arecaceae	X			X		X	X		X	X			
26	Palo perico	*Simarouba amara*	Simaroubaceae		X		X					X	X		X	
27	Pavonal	*Andropogum sp*	Poaceae			X	X							X		
28	Pavonilla	*Glossoloma panamense*	Gesneriaceae		X		X				X					
29	Pos	*Spathiphyllum sp*	Araceae	X			X				X		X			
30	Potranca	*Senna occidentalis*	Fabaceae	X			X						X			
31	Quitasol	*Mauritella pacifica*	Arecaceae	X					X		X		X			
32	Resucito	*Hibiscus* sp	Malvaceae	X			X									X
33	Tasi	*Cyathea sp*	Cyatheaceae	X				X		X	X	X	X		X	
34	Vaina	*Quararibea sp*	Malvaceae	X			X			X	X		X			
35	Virgusa	*Cecropia virgusa*	Urticaceae	x												X
36	Yerba dulce	*Borreria sp*	Rubiaceae	X			X							X		
37	Yogo	*Bidens* sp	Asteraceae	X			X									X
38	Zapallito	*Cucurbita sp*	Cucurbitaceae	X			X			X			X			

OJ = Separation of gold and jagua, N = Blackening troughs, G = Eliminating grease in the gold.
Part of the vegetation. H = Leaf, T = Stem, R = Root, S = Sap.
Location of the Information: C = Cértegui, T = La Toma, A = Ánimas, AG = Aguaclara, RL= Recta Larga, Q= Quiadó.

Table 4. Vegetation species used to create tools by community.

Artisanal Tools	Total of species	Species by community					
		Certegui	La Toma	Recta Larga	Animas	Agua clara	Quiado
Troughs	14	10	5	5	1	11	10
Cachos	13	11	6	5	1	9	11
Tool Handles	28	13	7	7	3	14	20
Storing gold (Totumo)	1	1	1	1	1	1	1
Weighing gold (balances)	2	1	1	2	2	1	2

Table 5. Vegetation species used in the practices of traditional mining for the purpose of correction.

Types of traditional mining	Total of species	Species by correction					
		Certegui	La Toma	Recta Larga	Animas	Agua clara	Quiadó
Mazamorreo	3	3	2	2	1	2	2
Holladero	17	14	7	10	1	6	11
Guache o Socavón	23	20	13	10	1	9	16
Zambullidero	1	0	0	0	1	1	1

Table 6. Volume of the substance and amount of time required to separate gold from jagua.

Species	Sample weight (oz.)	Volume of water (mL)	Volume of vegetation substance used in separation (mL)	Time used (min)
Hibiscus rosa-sinensis	4	300	40	5
Sida rhombifolia	4	300	40	4.35
Pavonia fruticosa	4	300	40	1.18
Ochroma pyramidale	4	300	40	1.45
Cecropia peltata	4	300	40	5.28
Apeiba tibourbou	4	300	40	2.35

The process of separating the jagua from the gold requires the following steps: first the vessel is filled with the gold and platinum material. Second it is washed by rotation with water to classify the coarse material until obtaining the fine material conformed of jagua and gold to initiate the application of the mucilaginous substance, the data is presented in the previous table.

CONCLUSIONS

In traditional mining practices in the municipalities of the Panamerican Union and Certegui in the department of Chocó, Colombia, 78 vegetation species are being used for the extraction of gold in all mining activities.

It is relevant that mining communities take advantage of plant species for two important mining activities: the separation of gold from the jagua and the development of their artisanal tools.

According to the tests and the information collected, there are 27 vegetation species which serve to separate gold from jagua, leaving it totally clean and does not require

to be amalgamated when at least 27 vegetation species are used for this purpose, replacing the use of mercury.

The mining community reports that the plant species Cedro Macho (*Tapirira guianensis*) is the most used in the production of artisanal troughs, a tool used ancestrally in barequeo mining.

The plant species Hibiscus rosa-sinensis, *Hibiscus rosa-sinensis, Sida rhombifolia, Pavonia fruticosa, Ochroma pyramidale, Cecropia peltata* and *Apeiba tibourbou* are the most used in the separation of gold and jagua.

Therefore, this work is novel, since the tests were carried out with the active participation of the community where the experience originated, who confirm the effectiveness of the separation and generate questions for the continuity of this investigative exercise.

ACKNOWLEDGEMENTS

To the traditional miners of Certegui and the Pan-American Union for their willingness to share their ancestral knowledge and especially to the members of the communities of Certegui Cabecera, Toma, Strata Long, Animas Cabecera, Quiadó, Agua Clara, as well as members of the Senior Community Councils of COCOMACER and COCOPAUPA, to make their installed capacity available and facilitate the process. To the social worker Sinlly Milena Aguilar, a native of the region, for her availability and companionship in the activities of connecting and collecting the information. To the technical team of CODECHOCO, for their participation and support. To the government of the department of Chocó for their support though the General System of Royalties. To the Technological University of Chocó, for their management, comprehension and designating us as teachers with the responsibility of developing the investigative process.

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Review article

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Revista Facultad Nacional de Agronomía

Plant responses to pathogen attack: molecular basis of qualitative resistance

UNIVERSIDAD NACIONAL DE COLOMBIA

Respuestas de las plantas al ataque de patógenos: bases moleculares de la resistencia cualitativa

doi: 10.15446/rfna.v70n2.64526

Kelly Ávila Méndez[1] and Hernán Mauricio Romero[2*]

ABSTRACT

Keywords:
Host resistance
Non-host resistance
Zig-zag model
PTI
ETI
ETS

Pathogens attack plants to assimilate nutrients from them. All plant species have succeeded in overcoming pathogenic attack; therefore disease condition is not the rule but the exception. A co-evolutionary battle has equipped plants with sophisticated defense mechanisms and cognate pathogens with a corresponding arsenal of counter strategies to overcome them. Traditionally, plant-pathogen interaction has been associated with molecules involved in recognition processes giving rise to models such as the "zig-zag Model". However, this model is being re-evaluated because it is not consistent with the complexity of the interaction. Current models propose a holistic view of a process where the response is not always determined by the interaction of two molecules. This review discusses the main aspects related to qualitative responses in the plant-pathogen interaction and the new proposed models.

RESUMEN

Palabras claves:
Resistencia hospedero
Resistencia no hospedero
Modelo zig-zag
PTI
ETI
ETS

Los patógenos atacan las plantas en un intento de asimilar los nutrientes de éstas. Todas las especies de plantas han tenido éxito para superar el ataque de patógenos, tanto que la condición de enfermedad no es la norma sino la excepción. Una batalla co-evolutiva ha dotado a las plantas con mecanismos de defensa sofisticados y a los patógenos afines con un arsenal correspondiente para superar dichas respuestas de defensa. Tradicionalmente, la interacción planta-patógeno se ha asociado a las moléculas que están involucradas en los procesos de reconocimiento, permitiendo el desarrollo de modelos que explican esta interacción, como el “Modelo zig-zag”. Sin embargo, éste modelo está siendo revaluado debido a que no es consistente con la complejidad de la interacción. Los modelos actuales proponen una visión holística de un proceso en el que no siempre la respuesta va estar determinada por la interacción de dos moléculas. Esta revisión discute los principales aspectos relacionados con la respuesta cualitativa en la interacción planta-patógeno y los nuevos modelos biológicos propuestos.

[1] Corporacion Centro de Investigaciones de Palma de Aceite, Cenipalma. AA 252171, Bogotá, Colombia.
[2] Facultad de Ciencias. Universidad Nacional de Colombia. AA 055051, Bogotá, Colombia
* Corresponding author <hmromeroa@unal.edu.co>

Received: April 19, 2016; Accepted: March 21, 2017
Rev.Fac.Nac.Agron. 70(2): 8225-8235. 2017
ISSN 0304-2847 / e-ISSN 2248-7026

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Plant diseases are a constant threat to agricultural production and thus to food security generating economic losses around the world. Pathogens that cause disease in plants include viruses, fungi, oomycetes, protozoa and nematodes (Pais *et al*., 2013; Bigeard *et al*., 2015). Traditionally the insects are excluded from this list; Agrios (2005), did not include them, but, actually the insects have been incorporated in the list of pathogens because there are evidences that the damage caused by insects could trigger a defense response similar to the one produced by common pathogens (Bever *et al.,* 2015; Conrath *et al.,* 2015; Stuart, 2015).

Global food security involves: food availability (production), access to food, and its utilization (for example nutritional aspects). At the same time, food security, presents a major imbalance in terms of growth and demand for food and world population. Thus, crop protection in terms of control of pests and diseases is a feasible strategy to reach the goals for food security (Savary *et al*., 2012, Mattews *et al*., 2013; Poppy *et al*., 2014).

The traditional approach to control and respond to plant diseases has involved the use of pesticides, which, apart from the cost to the environment and human health, is not always able to reduce the incidence of the disease. Therefore, even with the application of pesticides, crop losses continue to occur, causing high production costs, poor quality of products, and higher costs for the end consumer (Godfray *et al*., 2010; Ronald, 2011; Fu and Dong, 2013; Lapin and Van den Ackerveken, 2013; Li *et al*., 2013).

Due to the population growth estimated to reach 9 billion by 2050, the demand for food is high and so it is necessary to develop new varieties able to produce under limiting conditions such as high temperatures, water deficit, salinity and biotic stresses (Ronald, 2011; Mba *et al*., 2012).

Climate change will have a direct impact on the incidence of pests and diseases of crops -affecting between 12% and 13% of crop yields. Bebber and Gurr (2015), suggest that it is necessary to build a general framework for understanding the dynamics of plant communities at the large-scale, in order to generate predictions of change in plant communities over time. Such a framework would need to incorporate the environmental dependence of plant–pathogen interactions and plant–pathogen coevolution. These two features are particularly important in the face of climate change.

For example, in Colombia, in crops such as coffee, the increasing drought periods have been responsible for outbreaks in pests like the berry borer (Mba *et al*., 2012). The following table shows some important diseases in different crops in Latin America to 2013.

Table 1. Principal diseases that affect important crops in Latin America.

Organisms	Scientific name/ Name of the disease	Affected crop
Fungus	*Hemileia vastatrix /Coffee leaf rust*	Coffee*
	Phakopsora pachyrhizi / Rust	Soybean
	Mycosphaerella fijiensis / Sigatoka negra	Banana*
	Moniliophthora roreri/ Frosty pod rot	Cacao*
Oomycetes	*Phytophthora palmivora*/Bud rot	Oil Palm*
	Phytophthora ramorum/ Sudden Oak death	Oak
Bacteria	*Burkholderia glumae/Panicle blight*	Rice*
	Candidatus Liberibacter americanus /Yellow Shoot or HLB	Citrics
Virus	Barley yellow dwarf luteoviruses (BYDV)	Wheat
	Banana bunchy topo nanovirus (BBTV)	Banana
	Tomato yellow leaf curl begomovirus (TYLCV)	Tomato

*Diseases present in Colombia

Latin America is a highly diverse region in its ecosystems and the future of the biodiversity, and its associated ecological services depend on the ability to find a balance between conservation and development goals (Balvanera *et al*., 2012; Mujica and Kroschel, 2013; Lee *et al*., 2014; Bebber and Gurr, 2015). In the case of Colombia, 37.3% of its area has an agricultural use, however, under a climate change scenario new diseases and pests would emerge and it is necessary to find alternative strategies for crop health management.

Genetic breeding is an old agricultural activity; humankind has domesticated plants and animals to increase yields and productivity. The main target for breeding has been the increase in production, and to achieve it, the effective control of pests and diseases its mandatory. Conventional breeding takes a long time to obtain an improved variety, however, techniques as induced mutagenesis or transgenesis have had a big impact on the production of crops, by speeding up the breeding process, and the production of new varieties through these non-conventional methods. Unfortunately, technical, economical and society problems are imposing new challenges for the use of these biotechnological tools for producing the new varieties.

Genome editing has emerged as a new tool of low cost, low environmental impact and high effectiveness to improve the quality of crop production. However, as in the traditional biotechnology (mutagenesis, transgenesis) one important requirement is the knowledge of the metabolic networks, and the identification of specific targets (genes) (Ma *et al*., 2015; Quetier, 2016). There are numerous publications on genome editing (this review does not seek to cover them), but again, for the specific case of resistant to diseases, it is necessary to continue the efforts to understand the plant-pathogen relationships and to identify the key players (genes) on the pathogenicity (pathogen side) and resistance (plant side) (Beljah *et al*., 2015).

This review will focus on the molecular response of plants to the presence of a pathogen, describing the molecular aspects of qualitative resistance. The models proposed to date are also discussed due to the high complexity of the biology of plant-pathogen interactions.

The plant immune system

Plant-pathogen interactions can be considered as a two-way communication processes in which not only the plant is able to recognize a foreign organism and defend itself from it, but the pathogen must also be able to manipulate the biology of the plant to create an optimal environment for its own growth and development avoiding the plant response (Pritchard and Birch, 2011; Smale, 2012; Boyd *et al*., 2013; Kushalappa *et al*., 2016).

Plants, unlike animals, lack a defined immune system. They rely on the innate immunity of each cell and the systemic signals occurring at infection sites (Schulze-Lefert and Panstruga, 2011; Bonardi *et al*., 2012; Lapin and Van den Ackerveken, 2013). However, with the particular characteristics of the plant defense system, the molecular mechanisms used by these organisms are similar to animals (Zipfel, 2014; Chiang and Coaker, 2015; Keller *et al.,* 2016).

Plant pathogens, in general, are divided into biotrophic and necrotrophic pathogens, although there is a third group, called hemibiotrophic (Spoel and Dong, 2012; Okmen and Doehlemann, 2014). The difference between these groups lies in their lifestyle. Biotrophic pathogens obtain their nutrients from living host tissue, while necrotrophic pathogens obtain their nutrients from dead host tissue, and for this reason the mechanisms used by each kind of pathogens to infect a plant is different (Koeck *et al*., 2011; Lai and Mengiste, 2013).

Hemibiotrophic pathogens combine two strategies; they have an initial phase -biotrophic- in which the pathogen must evade the recognition from the host. This phase is followed by a necrotrophic stage in which toxins are secreted by the pathogen to induce host cell death; typically the visual symptoms start in this phase. Because of the presence of the asymptomatic biotrophic phase, the infection process with hemibiotrophic pathogens is difficult to understand and describe (Lee and Rose, 2010; Koeck *et al*., 2011; Vleeshouwers and Oliver, 2014).

The genetic basis of plant resistance to pathogens is divided into qualitative resistance (monogenic resistance) and quantitative resistance (polygenic resistance) (Lopez, 2011). Qualitative resistance can be explained by the *gene-for-gene* model proposed in the fifties by Flor (1971), who determined the basis of inheritance of resistance to flax rust in flax cultivars. According to the type of interaction

with the pathogen, plant responses at the molecular level are divided into two types: i) non-host response and ii) host response. These two types of responses which differ mainly by the molecule type (both from the plant and the pathogen), involved in the process (Maekawa *et al*., 2011; Schmidt and Panstruga, 2011; Li *et al*., 2013) will be explained in the next two sections.

Non-host resistance

Non-host resistance, is defined as the event where a plant species in particular is resistant to different kind of pathogens (either bacteria, fungi, oomycetes, or viruses) but, these same pathogens can infect other plant species (Bent and Mackey, 2007; Fan and Doerner, 2012; Bellincampi *et al*., 2014).

This response spectrum is caused by specific recognition processes between pathogen molecules called MAMPs (microbe-associated molecular patterns), which are recognized by PRR-type (pattern recognition receptors) membrane receptor proteins. PRR structures are types of receptor-like kinases (RLKs) that have functional modular domains (Beck *et al*., 2012; Monaghan and Zipfel, 2012; Wu *et al*., 2014a). However, MAMPs are molecules involved not only in the pathogenesis process; most of them, in fact, have been described as essential components of the cell, such as flg22. There are other types of MAMPs such as the HAMPs (herbivore-associated molecular patterns) and the DAMPs (damage-associated molecular patterns; originally called endogenous elicitors), but in general most literature talk about MAMPs to refer to this kind of molecules (Conrath *et al*., 2015).

The molecular response triggered by the recognition of MAMPs is known as PTI (*PAMP-triggered immunity*) in which some molecular mechanisms associated with PTI include: production of ROS, Ca^{+2} cascades, and the activation of MAPK (*mitogen-activated protein kinases*) cascades, involving Ca^{+2}-dependent proteins that ultimately lead to transcriptional reprograming (Bernoux *et al*., 2011; Uma *et al*., 2011; Yoshioka *et al*., 2011; Bigeard *et al*., 2015; Trapet *et al*., 2015). Well-known case-studies include the following:

Bacterial PAMPs

- FLS2 receptor (receptor-like kinase flagellin sensing 2) of *Arabidopsis thaliana* that interacts specifically with the oligopeptide flg22 of Gram-negative bacteria (Lu *et al*., 2010; Albrecht *et al*., 2012; Wang, 2012).
- EFR receptor (*Ef-Tu receptor*) recognizes the oligopeptide elf18. A particular characteristic that has been described only for plant species of the *Brassicaceae* family (Beck *et al*., 2012; Wang, 2012).
- Receptor XA21: Identified in rice, associated with specific resistance to various bacterial strains of the *Xanthomonas oryzae* pv. *oryzae* species (Lee *et al*., 2009).

Fungal and oomycetes PAMPs

- CeBIP and CERK1 receptor: LysM domains were initially identified as carbohydrate-binding domains in bacteria (Monaghan and Zipfel, 2012). Evidence that these domains (LysM- RLKs) are involved in PTI activity come from their identification in rice (CeBIP) and in *Arabidopsis* (CERK1) (Miya *et al*., 2007; Macho and Zipfel, 2014), where these domains bind together and specifically recognize chitin fragments.
- EiX1 and EiX2 receptors: EIX (*ethylene-inducing xylanase*) proteins which induce ethylene synthesis and PR (*pathogen-related proteins)* gene expression, are plant elicitors identified in tobacco and tomatoes. Their action is associated with a HR response (Ron and Avni, 2004).
- Cf-9: identified in tomato was the first protein LRR-RLP and confers resistance to the fungus *Cladosporium fulvus).*

In the case of oomycetes, the PAMPs and the PRR have not been described yet. There are reports with some approximations like the soluble beta- glucan-binding protein (GBP) from soybean (*Glycine max*) that recognizes heptaglucosides from the oomycete *Phytophthora sojae.* Additionally, other PAMPs have been identified that can trigger immune signaling in fungi or oomycetes. For example, plants can recognize fungal ergosterol and other oomycete PAMPs including arachidonic acid, elicitins, the transglutaminase-derived immunogenic epitope Pep13. However, in all of these cases, the PRR proteins have not been identified so far (Zipfel, 2014).

This first line of defense response is usually effective against some pathogens, however, the pathogen has adapted its molecular infection mechanisms to evade or suppress the PTI, secreting proteins (called effectors), that

trigger what is known as a host resistance mechanism (Monaghan and Zipfel, 2012; Couto and Zipfel, 2016). This plant-pathogen interaction has been explained by the zig-zag Model proposed by Jones and Dangl (2006) which suggests a co-evolutionary response process involving two plant immunity branches (PTI and ETI) and a transition phase (ETS). The details about this model will be described in the final section.

In addition to the mechanisms of PTI previously described, new evidence suggests that plants also use RNA silencing mechanisms as a defense mechanism. In this case, the plant has specific miRNA sequences that regulate gene expression and defend cells against invasive "nucleic acids", whether they are transposons, transgenes or viruses (Zvereva and Pooggin, 2012).

The first miRNA identified to be involved in PTI was miR393 in *Arabidopsis thaliana* in response to *Pseudomonas syringae*. miR393 is induced by flg22 and then suppresses auxin signaling by negatively regulating mRNAs of auxin receptors, transport inhibitor response 1 (TIR1), AFB2 and AFB3, which allow plants to prioritize defense signaling over plant growth, and trigger a series of defense responses. The role of miRNAs in PTI has also been demonstrated in fungal and oomycete infection, for example, osa-miR7695 was found to accumulate in rice treated with blast fungal mycelia (Fei *et al.,* 2016; Huang, 2016).

Host resistance

Molecular events during ETI (effector-triggered immunity) processes overlap with PTI. This branch of plant immunity occurs within the cell and originates once the host recognizes the effectors secreted by the pathogen, in which plant resistance (R) proteins can perceive these effectors initiating a defense response, including oxidative burst, accumulation of hormones such as salicylic acid (SA) and NOI (nitrogen oxide), MAPK cascades, changes in calcium levels, transcriptional reprogramming and synthesis of antimicrobial compounds, expression of *pathogeneses related* (PR) genes (Hein *et al*., 2009; Coll *et al*., 2011; De Bruyne *et al*., 2014; Stael *et al*., 2015).

Depending on the type of pathogen, the plant can induce programmed cell death (PCD) processes, also termed hypersensitive response (HR). These processes seek to block the advance of biotrophic pathogens to avoid an infection in different host tissues. In this process, chloroplasts play a key role in the production of ROS-type molecules and NOI (Robert-Seilaniantz *et al*., 2007; Lopez, 2011; Presti *et al*., 2015). For necrotrophic pathogens, the plant cell wall is the first line of defense providing a dynamic interface for interaction with necrotrophic pathogens. The interaction includes serving as a rich source of carbohydrates for the growth of pathogens; acting as a physical barrier to restrict the progression of the pathogens, and playing a role as an integrity sensory system that can activate intracellular signaling cascades in which plant hormones like jasmonic acid (JA) and ethylene (ET) play a major role in the defense response against these pathogens (Vleesschauwer *et al.,* 2014).

R proteins and activation

R proteins recognize effectors and activate the defense-signaling network (Hogenhout *et al*., 2009; Song *et al*., 2009; Gururani *et al*., 2012). Generally, this type of resistance confers complete and specific resistance; that is why it is also called race-specific resistance (de Jonge *et al*., 2011; Saintenac *et al*., 2013). The R proteins are codified by NB-LRR genes, one of the largest and most variable gene families found in plants (Collier and Moffett, 2009).

Most R proteins belong to a subgroup of a family of proteins called STAND (*signal transduction ATPase with numerous domains)*. NBS-LRR (*nucleotide-binding site; leucine rich repeats)* proteins that are subdivided into two subclasses depending on their N-terminal domain, -TIR- (*Toll/Interleukin-1 receptors)* domain or -CC- (*coiled coil)* domain, and are known as NBS-LRR-TIR and NBS-LRR-CC, respectively (Marone *et al*., 2013; Wu *et al*., 2014a).

For signaling, the NBS-LRR-CC proteins generally required a GPI anchored protein named non-race specific disease resistance 1, while NBS-LRR-TIR proteins require an enhanced disease susceptibility 1 for signaling. Additionally, the NBS-LRR-CC proteins are found in dicots and monocots whereas NBS-LRR-TIR are restricted to dicots (Chiang and Coaker, 2015; Cui *et al.,* 2015). The mechanism that activates R proteins and the subsequent signaling cascade in ETI is still being debated. Related to recognition, the simplest model is the direct interaction model in which there is a physical interaction between the pathogen effector and the R protein. An example of this mode of interaction

occurs between the pita CC-NB-LRR immune receptor in rice and the AvrPita effector of the fungus *Magnaporthe grisea* (Liu *et al*., 2011).

There are no particular characteristics that distinguish the way in which these proteins can sense different classes of pathogens (Collier and Moffett, 2009). However, the recognition process could be modeled in a more complex way through an indirect recognition. This form of recognition has led to the development of alternative recognition models:

Guard hypothesis. The guard hypothesis suggests that R proteins can detect changes or alterations caused by the effector to the host "guard" protein. One of the cases reported for this model corresponds to the RIN4 (*RPM1 interacting protein 4)* protein of *A. thaliana,* which is associated with two CC-NB-LRR-RMP1 and RPS2-type proteins. RIN4 is the target protein for AvrRpm1 and Avrpt2 effectors which, because of their protease activity, induce cleavage of RIN4, and this cleavage is detected by R proteins (Caplan *et al*., 2008; Van der Hoorn and Kamoun, 2008).

Decoy hypothesis. The "decoy" protein mimics the pathogen effector target, so the decoy functions mainly to restrict the pathogen but is not involved in the immune response (van der Hoorn and Kamoun, 2008). This model has been discussed mainly from the evolutionary point of view, it is expected that in the presence of the *R gene*, natural selection favors the decoy protein, but in the absence of the *R gene*, natural selection will cause the protein to decrease its affinity for the effector (Saintenac *et al*., 2013; Wu *et al*., 2015). Van der Hoorn and Kamoun (2008), explain the new model and offer four study cases to explain the model as follows:

Plant-pathogen interaction models

As we previously explained, each immunity branch has specific molecules, but, knowing the molecules and the process that produce them is not the only information that we need to understand the plant immunity. The next section provides information about the zig-zag model that until today is still the most accepted model. However, there are updated or new versions of the model.

Zig-zag model. In the most basic interaction, the zig zag model involves an interaction between the pathogen and the host. The interaction can be divided in four phases:

- Phase 1: plants detect MAMPs via PRRs to trigger PAMP-triggered immunity (PTI).
- Phase 2: successful pathogens deliver effectors that interfere with PTI, resulting in effector-triggered susceptibility (ETS).
- Phase 3: an effector can be recognized by an NB-LRR protein, activating effector-triggered immunity (ETI), which after surpassing a defined threshold induces hypersensitive cell death (HR).
- Phase 4, pathogen strains that have lost certain effector are selected. They might have also gained a new set of effectors to respond to the plant defense.

This model is being reevaluated, as some authors argue that describing a pathosystem as a model of interaction between molecules is a reductionist view of a process that is clearly highly complex. Other authors express concerns regarding the confusion that could arise from the terms of avirulence genes, virulence genes and effectors (Cook *et al*., 2014; Pritchard and Birch, 2014). The intent of this debate is not to invalidate any model, but to draw attention to certain issues discussed in the opinion article by Pritchard and Birch (2014), who describe six limitations of the zig-zag Model:

1. Molecular approach: It does not include DAMP. Therefore, it is suggested that the model is restricted to interactions with biotrophic pathogens.
2. Environmental context: By excluding the environmental factor it eliminates the effects of the interaction of the environment with the species that could affect the activation or suppression of molecular processes.
3. Organization of interaction events: The authors suggest that interaction events do not occur in organized phases, but, on the contrary, they can be stochastic processes.
4. Timescale: A model without a timescale does not allow for an adequate explanation of Phase 4 of the model (Phase 4: Gain / loss of effectors).
5. Physical scale: As in point 4 above, there is no population context to which it must be subjected for the gain or loss of effectors.
6. Qualitative model

Authors like Fei and collaborators (2016) have completed the 4 phases of the model introducing the effect that miRNAs have on the response of ITP and TSI. For example, miR393, which targets genes that are involved

in auxin signaling (TIR1, AFB2, and AFB3) is induced upon treatment with flg22. The repression of auxin signaling during infection enhances host PTI by hormone crosstalk. Effectors from pathogens can suppress the levels of plant miRNAs, such as miR393, to enhance susceptibility. However, the miR482 family, a negative regulator of plant Resistance (R) genes, can also be repressed upon detection of effectors to enhance effector-triggered immunity (ETI). Thus, although the zig-zag model is still maintained in its most basic sense as far as lines of defense are concerned, nevertheless, it is accepted that depending on the pathosystem, the model could have more components and could be updated if necessary.

Invasion model. This model was proposed by Cook *et al*. (2015), the authors took into consideration some limitations of the zig zag model such as: the model is restricted in terms of what microbe-associated molecule patterns (MAMPs) the plants can perceive through pattern recognition receptors (PRRs).

The invasion model has been explained in a similar way than the the zig-zag model, except in the aspects related to the definition of the immunogenic molecules. The authors suggest that these molecules must be represented as a continuum, and they argue that these molecules play other roles beyond the pathogenicity. Thus, the evolution can affect these molecules and have effects in an interaction model. In this sense, if a molecule has a role in a different process some evolutive forces can alter them, producing changes in the interaction process or even in the fitness of the species.

Multicompent model. The model was proposed by Andolfo *et al*. (2016). Like in the previous model, the authors start showing the disadvantages of the zig-zag model such as the fact that the model only describes two perception layers (PTI and ETI).

The multicomponent model has two components: activation and modulation, and it is divided in three phases as follows:

1) Interaction: two principal effects are detected: i) modifications of virulence factor targets and ii) specific alterations of primary plant metabolism.
2) Activation: modifications of virulence factor targets induce the Nibblers Triggered Signaling (NTS) or PPRs Triggered Signaling (PTS), mediated by R-genes activation. Metabolic alterations induce a feedback regulation of primary metabolic pathways resulting in a Hormone Tempered Resistance (HTR).
3) Modulation or effective resistance stage, the NTS/PTS, and the HTR converge to confer a resistance specific to the lifestyle of pathogen (Pathogen lifestyle-Specific Resistance, PSR).

In this case, the authors try to emphasize that the model will be determined specifically by the pathogen´s life cycle. On the other hand, this model is in certain way connected with a new approach it which R genes and effectors are described like independent molecules, in a way that better articulate all the plant-pathogen related information for using molecular techniques to open a new era in crop breeding.

The two last models do not pretend to invalidate the original zig-zag model; the idea with these new perspectives is to bring a new concept in plant immunity with a holistic vision of a process that is not only related to a molecular interaction. Also, the model try to take into account other aspects that can affect the fitness of the pathogen and the host.

Final considerations

The advent of omics technologies has introduced a new and wide range of identification tools not only for one gene but also for areas of the genome that are associated with the pathogen response and the pathogen´s effectors (Brauer *et al*., 2014; Vayssier-Taussat *et al*., 2014; Thynne *et al*., 2015). Genomic studies should focus on identifying these areas of the genome, as they allow for visualizing the plant's response mechanisms and even making predictions about the evolution of these areas.

In silico approaches allow generating different views, not only at the species level but also at the population level, to describe, based on genome information, the evolution of plants to achieve durable resistance to diseases (Burdon and Thrall, 2014; Karasov *et al*., 2014; Knief, 2014; Brown, 2015).

As mentioned at the beginning of this review, the growing demand for food production is generating new challenges

to maintain crop production rates. Different authors suggest that genome editing technologies will allow to introduce or modify characteristics of interest that ensure high quality productions and quantity of different crops (Andolfo *et al.,* 2016; Baltes and Voytas, 2015; Hendel *et al*., 2015; Kole *et al*., 2015).

Three mechanisms for genome editing are recognized: ZFN (Zinc Finger Nucleases), TALEN (TAL effector nucleases) and CRISPR/Cas9. These technologies seek to modify the genome introducing DBS (double strand breaks), under the control of specific nucleases, such action may cause deletions, insertions or modifications in the genome that can result from the DNA repair mechanisms NHEJ (Non-homologous end joining) and HR (Homologous recombination) (Huang *et al.,* 2016; Mahesh, 2016; Nagamangala *et al*., 2015; Weeks *et al.,* 2016).

CRISPR/Cas9 is currently proposed as a simple and useful alternative for genome editing, since it allows the modification of multiple sites along the genome. Several case studies have been reported in plants using this technology (Fauser *et al.,* 2014; Jia and Nian, 2014; Lowder *et al*., 2015; Shan *et al.,* 2014). Weeks and collaborators (2014) present a complete review of the different case studies that have been developed in species such as *Arabidopsis thaliana*, wheat and rice using CRISPR-Cas9 technology, and it is expected that soon more and more advances in the breeding for disease resistance will come from the use of this technology.

The information available on plant-pathogen interactions has raised many questions. Certain questions are focused on the "simplicity" with which these very diverse mechanisms have been described, as we can see in the new models mentioned. The dynamic and comprehensive approach is what is now referred to as biological dynamic networks, where different aspects of the model, even evolutionary aspects, must be taken account (Pritchard and Birch, 2011; Niks *et al*., 2015).

According to Vleesschauwer *et al.* (2014), studies in rice are providing new insights, often revealing unique complexities, for this reason the description of a biological process is not only related to the molecules per se, it is necessary to analyze the organism to describe how these plant-pathogen relationships can modulate the interaction in a niche and in a population.

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ÍNDICE DE AUTORES

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

La Revista Facultad Nacional de Agronomía (RFNA), es una publicación de la Facultad de Ciencias Agrarias de la Universidad Nacional de Colombia - Sede Medellín. Esta orientada a profesores, investigadores, estudiantes, extensionistas y a todos aquellos profesionales que crean conocimiento y articulan la ciencia y la tecnología para hacer más productivo el campo a nivel empresarial y de economía campesina.

La periodicidad de la Revista es semestral, con circulación nacional e internacional y tiene como objetivo divulgar artículos escritos en inglés, originales e inéditos de carácter científico que respondan a preguntas específicas y que proporcionen soporte y pruebas a una hipótesis, en aspectos relacionados con las Ciencias Agronómicas, Zootecnia, Ciencias Forestales e Ingeniería Agrícola y de Alimentos y otras afines que contribuyan a la solución de los limitantes del agro en el trópico.

Teniendo en cuenta los criterios considerados por Colciencias, la revista acoge documentos de las siguientes tipologías:

Artículos de investigación científica y tecnológica: Documento que presenta, de manera detallada, los resultados originales de proyectos terminados de investigación. La estructura generalmente utilizada contiene cuatro partes fundamentales: Introducción, metodología (materiales y métodos), resultados y discusión, y conclusiones.

Artículos de revisión: Documentos producto de una investigación terminada donde se analizan, sistematizan e integran los resultados de investigaciones publicadas o no publicadas, sobre un campo en ciencia o tecnología, con el fin de dar cuenta de los avances y las tendencias de desarrollo. Se caracteriza por presentar una cuidadosa revisión bibliográfica de por lo menos 50 referencias.

Artículos de reflexión: Documento que presenta resultados de investigación terminada desde una perspectiva analítica, interpretativa o crítica del autor, sobre los temas específicos ya citados, recurriendo a fuentes originales.

Artículos cortos: Documento breve que presenta resultados originales preliminares o parciales de una investigación científica o tecnológica, que por lo general requieren de una pronta difusión. Para todos los casos el 60% de las citas debe provenir de artículos publicados en los últimos diez años.

Los artículos deben ser presentados de acuerdo a los lineamientos establecidos en las “Instrucciones a los Autores”; quienes incumplan las normas básicas no iniciarán el proceso editorial. Se debe diligenciar el formato “Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales”, el cual será suministrado por la Revista. Dicho documento es explícito en mencionar que todos los autores están informados y de acuerdo con someter el artículo a consideración de la Revista, que no hay conflictos de interés entre ellos y expresa que el contenido del manuscrito no ha sido ni será enviado para su publicación a otra Revista.

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INSTRUCCIONES A LOS AUTORES

Lineamientos generales

Los artículos pueden ser enviados al correo electrónico: rfnagron_med@unal.edu.co, o a través del Open Journal System en el Portalde Revistas de la Universidad Nacional de Colombia http://www revistas.unal.edu.co/. Sólo serán considerados artículos escritos en inglés. Adjunto se debe remitir el formato "Autorización para Publicación de Obras y Cesión de Derechos Patrimoniales", en el cual se acepta la no postulación simultánea del artículo a otras revistas u órganos editoriales y se ceden los derechos a la Revista para su difusión. Las formas de publicación son: artículos de investigación científica y tecnológica, artículos de revisión, artículos de reflexión y artículos cortos. Los artículos pueden ser elaborados por profesores y/o investigadores de la Universidad Nacional de Colombia, o cualquier otra institución afín, nacional o internacional, en los temas Agropecuarios, Forestales y de Ingeniería Agrícola y de Alimentos. La extensión no debe exceder de 5.200 palabras, las hojas deben ser tamaño carta, escritas a interlineado sencillo, letra o fuente Times New Roman o Verdana, tamaño 12 puntos, márgenes de 3 cm en la parte superior, 2 cm en la inferior y 2,5 cm en las márgenes laterales derecha e izquierda. Las tablas y figuras (es decir, los gráficos, dibujos, esquemas, diagramas de flujo, fotografías y mapas) se deben mostrar incorporadas en el texto y con numeración consecutiva (Tabla 1... Tabla n; Figura 1... Figura n, etc.). Los textos y tablas se deben presentar en el procesador de palabras MS-Word®;las tablas y los diagramas de frecuencia (barras y tortas) originales se deben suministrar en el archivo del documento y también en su original de MS-Excel®; otras figuras, como fotografías sobre papel y dibujos, se pueden enviar en original o escaneadas y ser remitidas en el formato digital de compresión JPG (o JPEG) preferiblemente con una resolución de 600 x 600 dpi (mínimo 300 dpi); es deseable que las fotografías originales sean enviadas como diapositivas. Como norma general, las tablas y figuras sólo se aceptan en blanco y negro; excepcionalmente se incluirán en color cuando sea estrictamente necesario y a juicio del Comité Editorial.

Unidades, abreviaturas y estilo

Se debe utilizar el Sistema Internacional de Unidades (SIU), y aquellas unidades específicas de mayor uso por parte de la comunidad científica. Cuando se requiera se debe usar la forma exponencial. Ejemplo: kg ha^{-1}. El significado de las abreviaturas debe citarse por extenso cuando se mencionan por primera vez en el manuscrito. El estilo de escritura debe ser absolutamente impersonal, en tiempo gramatical pasado para la introducción, los procedimientos y los resultados y presente para la discusión, evitando la conjugación de verbos en primera o tercera persona del singular o el plural.

Los números del uno al nueve se escriben en palabras, excepto cuando incluyen unidades de medida o se mencionan varios números. Ejemplo: "ocho tratamientos", "3, 7 y 9 lecturas", "15 kg". Use cero antes del punto decimal. Para separar números en intervalos de uno o más años, use la letra "a", y guión para temporadas de crecimiento. Ejemplo: Periodo 2002 a 2005; temporadas de crecimiento 1999-2000, 2000-2001.

Título y autores

El título del artículo no debe incluir abreviaturas y es obligatoria su respectiva traducción al idioma español. En lo posible, el título no debe exceder de 15 palabras y debe reflejar con precisión el contenido del documento. Cuando contenga nombres científicos de especies vegetales o animales, éstos se deben escribir con letra cursiva (itálica) en minúsculas, sólo con mayúsculas la primera letra del género y del clasificador. Debajo del título en inglés se escribe el nombre(s) y apellido(s) de los autores, sin sus respectivos títulos académicos, ni cargos laborales, en una línea horizontal y de acuerdo con su contribución en la investigación y/o preparación del artículo.

Como nota al pie de la primera página, se escribe el título de pregrado, el cargo laboral de los autores, el nombre y la ciudad de ubicación de la entidad a la cual prestan sus servicios o del patrocinador para la realización del trabajo y su respectiva dirección de correo electrónico, indicando el autor de correspondencia. Además, se debe adjuntar un resumen de la hoja de vida de los autores, donde se mencionen los artículos publicados en otras revistas.

Resumen, abstract y palabras claves

El resumen no debe exceder de 250 palabras escritas en un único párrafo. Se debe escribir en inglés y español. Debe contener en forma breve la justificación, los objetivos, los métodos utilizados, los resultados obtenidos más relevantes y las conclusiones. Es obligatorio acompañar el resumen con un máximo de seis palabras clave distintas a las utilizadas en el título. Se aceptan como palabras clave no sólo las palabras simples, sino también términos compuestos hasta de tres palabras. Deben ir escritas en minúsculas y separadas por comas.

Introducción

Puede tener o no título. Define el problema e informa sobre el estado del arte respecto al tema principal del artículo; además, señala las razones que justifican la investigación y plantea los objetivos de la misma. Es obligatorio acompañar los nombres vulgares con el nombre(s) científico(s) y la abreviatura(s) del clasificador en la primera mención dentro del texto. No se deben mencionar marcas de productos, sino su nombre genérico o químico

Materiales y métodos

En este apartado se deben describir en forma clara, concisa y secuencial, los materiales (vegetales, animales, implementos agrícolas o de laboratorio) utilizados en el desarrollo del trabajo; además, se mencionan los aspectos relacionados con la ubicación, preparación y ejecución de los experimentos. Se debe indicar el diseño seleccionado, las variables registradas, las transformaciones hechas a los datos, los modelos estadísticos usados y el nivel de significancia empleado. Evitar detallar procedimientos previamente publicados.

Resultados y discusión

Son la parte central del artículo, deben estar respaldados por métodos y análisis estadísticos apropiados. Se deben presentar de manera lógica, objetiva y secuencial mediante textos, tablas y figuras; estos dos últimos apoyos deben ser fáciles de leer, autoexplicativos y estar siempre citados en el texto. Las tablas se deben elaborar con pocas columnas y renglones. Se debe tener

la precaución de incluir el nivel de significancia estadística representado por letras minúsculas del comienzo del alfabeto (a, b, c, d,...), un asterisco simple (*) para *P*<0,05, doble asterisco (**) para P<0,01 o triple asterisco (***) para *P*<0,001. Las investigaciones que no siguen un diseño estadístico, deben mostrar la información de manera descriptiva. Use subíndices para modificaciones, reserve superíndices para potencias o notas al pie en tablas y figuras.

La discusión Se refiere al análisis e interpretación objetiva de los resultados, confrontándolos con los obtenidos en otras investigaciones, o con los hechos o teorías conocidos sobre el tema. Explica los resultados en particular cuando difieren de la hipótesis planteada. Destaca la aplicación práctica o teórica de los resultados obtenidos y las limitaciones encontradas. Resalta la contribución que se hace a una determinada área del conocimiento y el aporte a la solución del problema que justifica la investigación. Finalmente, proporciona elementos que permitan proponer recomendaciones o lanzar nuevas hipótesis. No se deben hacer afirmaciones que van más allá de lo que los resultados pueden apoyar.

Conclusiones

Son las afirmaciones originadas a partir de los resultados obtenidos, deben ser coherentes con los objetivos planteados y la metodología empleada; además, expresar el aporte al conocimiento en el área temática estudiada y proponer directrices para nuevas investigaciones.

Agradecimientos

Si se considera necesario, se incluyen los agradecimientos o reconocimientos a personas, instituciones, fondos y becas de investigación, que hicieron contribuciones importantes en la concepción, financiación o realización de la investigación.

Literatura citada

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

La bibliografía se deberá incluir al final del texto, sólo con las referencias citadas en el mismo. Se debe incluir el número doi asignado a cada artículo consultado. Las citas en el texto deben incluir apellido del autor y año, con coma entre autor y año. Ejemplo: Pérez, 1995; además conservar el siguiente orden de citación:

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- Si hay dos autores se citarán separados por la conjunción y. Ejemplo: Gil y Ortega, 1993.
- Si hay varios trabajos de un autor publicados en un mismo año, se citarán con una letra en secuencia alfabética de los títulos, adosada al año. Ejemplo: Gómez, 2000a, 2000b, 2000c.

En el caso de citas con tres o más autores, es necesario mencionar en el texto el apellido del primero y reemplazar los demás por la expresión latina abreviada *et al.* que significa y otros; en la bibliografía se deben citar todos los autores.

Las comunicaciones personales, se deben citar al pie de la página y no se incluyen en la bibliografía.

Las referencias bibliográficas se deben ordenar alfabéticamente por el apellido del primer autor, sin numeración y sin sangría. Para citar varias publicaciones del mismo autor, de debe seguir el orden cronológico creciente, si son del mismo año, se debe seguir el orden alfabético de los títulos.

Las referencias deberán contener todos los datos que permitan su fácil localización. Las referencias se citan en el lenguaje de la publicación original.

En cada referencia para todos los autores cite primero el apellido seguido de la inicial del nombre sin puntos, separando autores con coma y espacio.

Ejemplos:

Para libros: Autor (es). Año. Título del libro, edición, ciudad de su sede, casa editora y, páginas consultadas (pp. # - #) o páginas totales (# p.). Ejemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

Para capítulos de libros: Autor (es). Año. Título del capítulo, páginas consultadas (pp. # - #). En: Apellidos y nombres de los compiladores o editores (eds.), título del libro, edición, casa editora y ciudad de su sede, páginas totales (# p.). Ejemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. En: Agrios G. (ed.). Fitopatología. Segunda edición. Editorial Limusa, México D.F. 400 p.

Para artículos de revistas: Autor (es). Año. Título del artículo, nombre completo de la revista volumen(número): página inicial–página final. doi. Ejemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

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

Tesis, trabajos de grado. Gómez C. 2004. Autoecología del Mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia. Trabajo de grado Ingeniería Agronómica. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Cita de cita. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica 9(2): 227- 232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tesis Magister en Bosques y Conservación Ambiental. Facultad de Ciencias Agrarias, Universidad Nacional de Colombia. Medellín. 46 p.

Suplemento de revista. Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52 Supl. 2: 195-201.

Para citas de internet: Autor (es). Año. Título del artículo. En: Nombre(s) de la publicación electrónica, de la página web, portal o página y su URL, páginas consultadas (pp. # - #) o páginas totales (# p.); fecha de consulta. Ejemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. En: Agricultura Tropical, http://agrotropical.edunet.es. 25 p.; consulta: noviembre 2003.

PUBLISHING POLICY

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

The National Faculty of Agronomy Journal (RFNA) is published by the Faculty of Agricultural Sciences of Universidad Nacional de Colombia – Medellín. It is aimed at teachers, researchers and students in agronomy, animal, and forestry sciences, food and agricultural engineering, agricultural advisers and at all those professionals who create knowledge and articulate science and technology to make the field more productive at business and rural economy levels.

The Journal publication is biannual, at national and international level. Its aim is to disclose original and unpublished articles of a scientific nature which respond to specific questions and provide support and testing of a hypothesis, related to agronomy, animal husbandry, forestry engineering, food and agricultural engineering, and related areas that contribute to the solution of the agricultural constraints in the tropics.

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

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Review articles: Documents resulted from a completed research systematizing, analyzing, and integrating the published or unpublished research findings, on a field of science or technology, in order to report the progress and development trends. It is characterized by a careful review of the literature of at least 50 references.

Critical reflection articles: A document presenting completed research results from an analytical, interpretive or critical author's point of view, on the specific issues already mentioned, using original sources.

Short articles: short paper presenting original preliminary or partial results of a scientific or technological research, which usually require a quick diffusion. In all cases 60% of references must come from articles published in the last ten years.

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

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INSTRUCTIONS TO AUTHORS

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Units, abbreviations and style

International System of Units (SI), and those specific units of greater use by the scientific community must be used. When required must be used the exponential form. Example: kg ha^{-1}. The meaning of abbreviations should be cited in full when first mentioned in the manuscript. The writing style should be totally impersonal. Introduction, procedures and results should be written in grammatical past tense. Discussion should be written in grammatical present tense, avoiding the conjugation of verbs in first or third person singular or plural.

The numbers from 1 to 9 are written in words, except when they include units of measure or several numbers are listed. Example: "eight treatments", "3,7 and 9 readings", "15 kg". Use zero before the decimal point. To separate numbers in intervals of one to two years, use the letter "a" and hyphen for growing seasons. Example period 2002a2005, growing seasons 1999-2000, 2000-2001.

Title and authors

The article should not include abbreviations and its translation into English is required. As far as possible, the title should not exceed 15 words and must accurately reflect the paper content. When the article contains scientific names of plants or animals, they should be written in italics in lower case, only the first letter of gender and classifier should be capital. Under the title in English the author or authors' name (s) and surname (s) is /are written, without academic degrees or job positions, in a horizontal line according to the contribution to research and / or preparation of the article.

As a footnote on the first page, write the title of undergraduate, authors' job positions, the name and city location of the entity to which they serve, or the sponsors for the research work and their respective email address. In addition, a summarized authors' résumé including reference to the articles published in other magazines should be attached.

Abstract and key words

The abstract should not exceed 250 words written in a single paragraph. It must be written in English, Spanish or Portuguese. It should contain in brief the justification, aims, methods used, the most relevant results, and conclusions. It is required to accompany the abstract with a maximum of six key words, translated into English, different from those used in the title. Single words as well as compound terms of up to three words are accepted as key words. They must be written in lowercase, separated by commas.

Introduction

It may or not have a title. It defines the problem and reports on the state of the art on the main subject of the article, it also points out the reasons for the research and sets out its aims. It is required to accompany common names with the corresponding scientific name (s) name and abbreviation (s) of the classifier at the first mention in the text. Brands must not be mentioned but the generic or chemical name.

Materials and methods

In this section, materials (crops, livestock, agricultural or laboratory implements) used in the development of work should be clearly, concisely and sequentially described. Aspects related to the location, preparation and execution of experiments should also be mentioned. The selected design, the recorded variables, the changes made to data, the statistical models used and the significance level used should be indicated. Authors must avoid detailing procedures previously published.

Results

They are the central part of the article and must be supported by appropriate statistical methods and analysis. They should be presented in a logical, objective and sequential way through texts, tables and figures; the latter two supports should be easy to read, self- explanatory and always quoted in the text. The tables should be composed by few columns and rows. Care should be taken to include the statistical significance level represented by lowercase letters of the beginning of the alphabet (a, b, c, d,…), a single asterisk (*) for $P<0.05$, double asterisk (**) for $P<0.01$ or triple asterisk (***) for $P<0.001$. Researches that do not follow a statistical design should display the information in a descriptive way. Use subscripts to modifications, reserve superscripts for potencials or footnotes in tables and figures.

Discussion

It refers to the analysis and objective interpretation of results, comparing them with those obtained in other researches, or with known facts or theories on the subject. It explains the results, especially when they differ from the stated hypothesis. It emphasizes the practical or theoretical application of the obtained results and constraints encountered. Discussion also highlights the contribution that is made to a particular area of knowledge and to the solution of the problem that justifies the research. Finally, it provides elements that allow making recommendations or launching new hypotheses. Statements that go beyond what the results may support should be avoided.

Conclusions

Conclusions are assertions arising from the obtained results. They should be consistent with the objectives stated and the methodology used. They should also express the contribution to knowledge in the studied subject area and propose guidelines for further researches.

Acknowledgements

If necessary, acknowledgements or recognitions to individuals, institutions, funds and research grants that made important contributions in the design, financing or carrying out of the research are included.

Cited Literature

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

The bibliography should be included at the end of the text, containing only the references cited in it, including the doi number. Citations in the text should include author's surname and year, with comma between author and year. Example: Pérez, 1995. They should also keep the following citation order:

- If more than one date, they are separated by commas: Example: Pérez, 1995, 1998, 2001.

- If there are two authors, they will be separated by the conjunction and. Example: Gil and Ortega, 1993.

If there are several works by an author, published in the same year, they will be cited with a letter in alphabetical sequence of titles, adjacent to year. Example: Gómez, 2000a, 2000b, 2000c.

For citations with three or more authors, it is necessary to mention in the text the surname of the first and replace the others by the Latin expression *et al.,* which means and others. All authors should be mentioned in the bibliography.

Personal communications should be cited at the bottom of the page and not included in the bibliography.

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

The references should be arranged alphabetically by first author, without numbers and without indentation. To cite several publications by the same author, follow the chronological up order, if they are of the same year, follow the alphabetical order of the titles.

References should contain all the data allowing to its easy location.

Examples:

For books: Author (s), year. Book title, edition, place of publication, publisher and pages consulted (pp. # - #) or total pages (# p.). Example: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

For book chapters: Author (s), year. Chapter title, pages consulted (pp. # - #). En: Surnames and names of the editors or publishers (eds.), book title, edition, publisher and place of publication, total pages (# p.). Example: Bernal H. 1996. Chapter 6: Evapotranspiración. pp. 112-125. In: Agrios G. (ed.). Fitopatología. Second Edition. Editorial Limusa, México D.F. 400 p.

For journals: Author (s), year. Article title, journal full name volume(number): page-page. Example: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. doi: 10.1093/treephys/24.4.387

Presentations in Memoirs of Congresses, seminars and symposia: García M. 1998. Geotechnical engineering and environmental protection. p. 65-94. In: Memorias IX Colombian Congress of Soil Science. Colombian Society of Soil Science. Santa Fé de Bogotá.

Theses and dissertations: Gómez C. 2004. Autoecología de mortiño (*Vaccinium meridionale* Swartz Ericaceae). Master's Thesis in Forestry and Environmental Conservation. Faculty of Agricultural Sciences. Universidad Nacional de Colombia. Medellín. 78 p.

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Journal Supplement: Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Journal of Tropical Biology 52 Suppl. 2: 195-201.

For internet citations: Author (s), year. Article. In: electronic publishing Name (s), the web page, portal or page name and its URL, pages consulted (pp. #) or total pages (# p.), date of consultation. Example: Arafat Y. 1996. Siembra de olivos en el desierto palestino. In: Tropical Agriculture, http://agrotropical. edunet.es. 25 p.; accessed: November 2003.

POLÍTICA EDITORIAL

REVISTA FACULTAD NACIONAL DE AGRONOMÍA

A Revista Facultad Nacional de Agronomía é uma publicação da Facultad de Ciencias Agrarias da Universidad Nacional de Colombia – Sede Medellín. Orienta-se a professores, pesquisadores, estudantes e a todos os profissionais que criam conhecimento e articulam a ciência e a tecnologia para fazer o campo mais produtivo no âmbito empresarial e da economia camponesa.

A periodicidade da Revista é semestral, com circulação nacional e internacional e seu objetivo é divulgar artigos originais e inéditos de caráter científico que respondam perguntas específicas e forneçam suporte e provas a uma hipótese, em aspectos relacionados com das Ciências Agronômicas, Zootecnia, Ciências Florestais e Engenharia Agrícola e de Alimentos e disciplinas afins que contribuam à solução dos limitantes do agro no trópico.

Levando em conta os critérios considerados por Colciencias, a revista considera documentos das seguintes tipologias:

Artigos de pesquisa científica e tecnológica: Documentos que apresentam, de forma detalhada, os resultados originais de projetos de pesquisa concluídos. A estrutura utilizada contém, geralmente, quatro partes fundamentais: introdução, metodologia (materiais e métodos), resultados e discussão, e conclusões.

Artigos de revisão: Documentos produto de uma pesquisa concluída onde são analisados, sistematizados e integrados os resultados de pesquisas publicadas ou não publicadas, sobre um campo em ciência e tecnologia, a fim de dar conta dos avanços e tendências de desenvolvimento. Caracteriza-se por apresentar uma cuidadosa revisão bibliográfica de pelo menos 50 referências.

Artigos de reflexão: Documentos que apresentam resultados de pesquisa concluída com uma perspectiva analítica, interpretativa ou crítica do autor, sobre os temas específicos antes mencionados, recorrendo a fontes originais.

Artigos curtos: Documentos breves que apresentam resultados originais preliminares ou parciais de uma pesquisa cientifica ou tecnológica, e que geralmente precisam de uma rápida difusão. Para todos os casos o 60% das citações deve provir de artigos publicados nos últimos dez anos.

Os artigos devem ser apresentados de acordo com os parâmetros estabelecidos nas “Instruções para os Autores”, aqueles que não seguirem as normas básicas não serão considerados para publicação. Deve preencher o formulário “Autorização para Publicação de Obras e Sessão de direitos” a qual será fornecida pela Revista. O formulário é explícito enquanto que todos os autores estão informados do envio do artigo para a Revista, além de estar de acordo com ele. Também o formulário indica que não se apresentam conflitos de interesse entre eles e expressam que o conteúdo do manuscrito não tem sido nem será enviado para a sua publicação em outra revista.

O Comitê Editorial, junto a uma equipe de editores asociados, avaliará o mérito científico do documento e o submeterá a avaliação na modalidade duplo-cego–isto é, tem- se estrito anonimato da relatoría- por dois árbitros especializados no assunto, um deles nacional e outro internacional, os quais entregarão seu ditame no formato estabelecido pela Revista.

O Comitê Editorial reserva-se o direito de aceitar ou não as colaborações. O ditame após o processo de revisão pode ser: aceito para publicação com nenhuma ou poucas modificações; aceito para publicação com modificações maiores segundo as observações dos avaliadores; reconsiderado para publicação se modificado substancialmente –neste caso será catalogado como material novo-; recusado para publicação. Caso o artigo for aceito, este será devolvido aos autores para correção e remitido novamente ao Diretor da Revista nos seguintes 30 dias calendário.

A impressão de gráficos, figuras ou fotografías em cor é opcional e tem custo adicional por página precisava de cem mil pesos colombianos (100 mil pesos). A redação da Revista reserva-se o direito de realizar modificações de forma no texto do artigo (títulos, resumos/abstracts, tabelas e figuras), embora, sempre que seja possivel, os autores serão consultados a respeito das mudanças introducidas.

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INSTRUÇÕES PARA OS AUTORES

Parâmetros gerais

Os artigos podem ser enviados ao endereço eletrônico: rfnagron_med@unal.edu.co ou também ingressando no site das Revistas da Universidad Nacional de Colombia usando o programa Open Journal System http://www.revistas.unal.edu.co/. Serão considerados apenas os artigos escritos em Inglés. Junto com o trabalho deverá encaminhar o formulário "Autorização para Publicação de Obras e Sessão de direitos" no qual se aceita a não postulação simultânea do artigo a outras revistas ou órgãos editoriais e cedem-se à Revista os direitos de difusão. As formas de publicação são: artigos de pesquisa científica e tecnológica, artigos de revisão, artigos de reflexão e artigos curtos. Os artigos podem ser elaborados por professores e/ou pesquisadores da Universidad Nacional de Colombia, ou qualquer outra instituição afim, nacional ou internacional, nos temas agropecuários, florestais, e de engenharia agrícola e de alimentos. A extensão não deve superar as 5.200 palavras, as folhas devem ser tamanho carta, escritas a duplo espaço, letra ou fonte Times New Roman ou Verdana, tamanho 12 pontos, margens de 3 cm na parte superior, 2 cm na inferior e 2,5 cm nas margens laterais direita e esquerda. As tabelas e figuras (isto é, gráficos, desenhos, esquemas, diagramas de fluxo, fotos e mapas) devem aparecer em folhas independentes e com numeração consecutiva (Tabela 1... Tabela n; Figura 1... Figura n. etc.). Os textos e tabelas devem ser apresentados no processador de palavras MS-Word®; as tabelas e diagramas de freqüência (gráficos de barras e de pizzas) originais devem aparecer tanto no arquivo do manuscrito quanto no original de MS-Excel®; outras figuras, como fotos sobre papel e desenhos, podem ser enviadas em original ou digitalizadas, e remitidas no formato digital de compressão JPG (ou JPEG) preferivelmente com uma resolução de 600 x 600 dpi (mínimo 300 dpi); é desejável que as fotos originais sejam enviadas como slides. Como norma geral, só serão aceitas tabelas e figuras em preto e branco; imagens coloridas serão incluídas só em caso estritamente necessário e a juízo do Comitê Editorial.

Unidades, abreviaturas e estilo

Deve utilizar-se o Sistema Internacional de Unidades (SIU), e aquelas unidades específicas de maior uso por parte da comunidade científica. Quando seja necesario debe-se usar a forma exponencial Exemplo: kg ha^{-1}. O significado das abreviaturas deve ser citado por extenso quando mencionadas por primeira vez no manuscrito. O estilo da escrita deve ser absolutamente impessoal, em tempo gramatical pretérito na introdução, procedimentos e resultados, e presente na discussão, evitando a conjugação de verbos em primeira ou terceira pessoa do singular ou do plural.

Os números de um a nove devem-se escrever em palavras, exceto quando refletem ou indicam unidades de medida ou se colocam vários números consecutivamente Exemplo: "oito tratamentos", "3, 7 y 9 leituras", "15 kg". Deve-se utilizar o zero antes do ponto decimal. Para separar intervalos de um o mais anos, deve-se usar a letra "a", e hífen para períodos de crescimento (safras). Exemplo. Período 2002 a 2005, safras 1999-2000, 2000-2001.

Título e autores

O título do artigo não deve incluir abreviaturas e é obrigatória sua tradução ao inglês. Sempre que possível, o título não deve superar as 15 palavras e deve refletir com precisão o conteúdo do documento. Em caso de conter nomes científicos de espécies vegetais ou animais, estes devem ir em itálica minúscula, com maiúscula somente a primeira letra do gênero e do classificador. Embaixo do título em inglês escreve-se o nome(s) e sobrenome(s) dos autores, sem seus títulos acadêmicos, nem cargos laborais, numa linha horizontal e conforme a sua contribuição à pesquisa e/ou preparação do artigo.

Na parte inferior da primeira página, como nota ao rodapé, escreve-se o cargo laboral dos autores, o nome e a cidade onde se localiza a entidade para a qual trabalham ou do patrocinador para a realização do trabalho e o correspondente endereço eletrônico. Adicionalmente, deve anexar-se um resumo do currículo dos autores, onde se mencionem os artigos publicados em outras revistas.

Resumo, abstract e palavras-chave

O resumo não deve superar as 250 palavras escritas num único parágrafo. Deve ser redigido em espanhol, inglês ou português. Deve conter em forma breve justificação, objetivos, métodos utilizados, resultados obtidos mais relevantes e conclusões. É obrigatório acompanhar o resumo com um máximo de seis palavras-chave, traduzidas ao inglês (key words), diferentes às utilizadas no título. Aceitam-se como palavras-chave não somente palavras simples, mas também termos compostos por até três palavras. Estas devem ir escritas em minúscula e separadas por vírgulas.

Introdução

O título não é obrigatório. Define o problema e informa sobre o estado da arte a respeito do tema principal do artigo, além disso, indica as razões que justificam a pesquisa e propõe os objetivos da mesma. É obrigatório acompanhar os nomes vulgares com o nome(s) científico(s) e a abreviatura(s) do classificador na primeira menção dentro do texto. Não mencionar marcas de produtos, mas nomes genéricos ou químicos.

Materiais e métodos

Aqui devem ser descritos em forma clara, concisa e seqüencial, os materiais (vegetais, animais, implementos agrícolas ou de laboratório) utilizados no desenvolvimento do trabalho, assim mesmo mencionam-se os aspectos relacionados com a localização, preparação e execução dos experimentos. Devem indicar-se o desenho escolhido, as variáveis registradas, as transformações feitas aos dados, os modelos estatísticos usados e o nível de significância empregado. Evitar detalhar procedimentos previamente publicados.

Resultados

São a parte central do artigo, devem ir respaldados por métodos e análises estatísticas apropriadas. Devem apresentar-se de maneira lógica, objetiva e seqüencial mediante textos, tabelas e figuras; estes dois últimos apoios devem ser de fácil leitura, interpretáveis de forma autônoma e ir citados sempre no texto. As tabelas devem conter poucas colunas e linhas. É preciso incluir o nível de significância estatística representado por letras minúsculas do começo do alfabeto (a, b, c, d,...), asterisco simples (*) para $P< 0,05$, duplo asterisco (**) para $P< 0,01$ ou três asteriscos (***) para $P<0,001$. As pesquisas que não obedecem um

desenho estatístico devem mostrar a informação de forma descritiva. Deve-se utilizar subíndice para modificações, os superíndices devem ser utilizados para potencias ou notas ao rodapé em tabelas e figuras.

Discussão

Refere-se à análise e interpretação objetiva dos resultados, confrontando-os com os resultados obtidos em outras pesquisas, ou com os fatos ou teorias conhecidas sobre o tema. Explica os resultados, particularmente quando diferem da hipótese proposta. Destaca a aplicação prática ou teórica dos resultados obtidos e as limitações encontradas. Ressalta a contribuição a uma determinada área do conhecimento e o aporte à solução do problema que justifica a pesquisa. Finalmente, proporciona elementos que permitem propor recomendações ou lançar novas hipóteses. Não devem ser feitas afirmações que vão além do que os resultados podem apoiar.

Conclusões

São as afirmações originadas a partir dos resultados obtidos, devem ser coerentes com os objetivos propostos e a metodologia empregada; adicionalmente, expressar a contribuição ao conhecimento na área temática estudada e propor diretrizes para novas pesquisas.

Agradecimentos

Caso for necessário, incluir-se-ão os agradecimentos ou reconhecimentos a pessoas, instituições, fundos ou bolsas de pesquisa que fizeram contribuições importantes na concepção, financiamento ou realização da pesquisa.

Literatura citada

Devem aparecer somente as referências bibliográficas mencionadas no texto. Não se aceitam notas de aula, artigos em construção ou no prelo, ou qualquer outra publicação de circulação limitada. Evitar o excesso de auto-citas.

A bibliografia deverá aparecer no final do texto, só com as referências citadas no mesmo. As citações no texto devem incluir sobrenomes do autor e ano, com vírgula entre autor e ano. Exemplo: Pérez, 1995; além de conservar a seguinte ordem de citação:

-Se houver mais de uma data, estas se separam com vírgula. Exemplo: Pérez, 1995, 1998, 2001.

-Se houver dois autores, estes se citam separados pela conjunção e. Exemplo: Gil e Ortega, 1993.

-Se houver vários trabalhos de um autor publicados no mesmo ano, estes se citam com uma letra em seqüência alfabética dos títulos, do lado do ano. Exemplo: Gómez, 2000a, 2000b, 2000c.

-Em caso de citações com três ou mais autores, é preciso mencionar no texto os sobrenomes do primeiro e substituir os outros pela expressão latina abreviada *et al.* que significa y outros; já na bibliografia devem aparecer citados todos os autores.

-As comunicações pessoais devem aparecer citadas no rodapé de página e não se incluem na bibliografia.

-As referências bibliográficas devem ir ordenadas alfabeticamente pelo sobrenome do primeiro autor, sem numeração e sem espaçamento na primeira linha. Para citar várias publicações do mesmo autor segue-se a ordem cronológica crescente, e no caso forem do mesmo ano seguirá a ordem alfabética dos títulos.

As referências deverão conter todos os dados que permitam sua fácil localização.

Exemplos:

Para livros: Autor(es), ano. Título do livro, edição, cidade de sua sede, casa editora e, páginas consultadas (pp. # - #) ou páginas totais (# p.). Exemplo: Robinson A, Morrison J, Muehrcke P, Kimerling AJ and Guptill S. 1995. Elements of Cartography. Sixth edition. John Wiley and Sons, Inc., New York. 674 p.

Para capítulos de livros: Autor(es), ano. Título do capítulo, páginas consultadas (pp. # - #). Em: Sobrenomes e nomes dos compiladores ou editores (eds.), título do livro, edição, casa editora e cidade de sua sede, páginas totais (# p.). Exemplo: Bernal H. 1996. Capítulo 6: Evapotranspiración. pp. 112-125. Em: Agrios G. (ed.). Fitopatología. Segunda edição. Editorial Limusa, México D.F. 400 p.

Para revistas: Autor(es), ano. Título do artigo, nome completo da revista (volume)número: página-página. Exemplo: García S, Clinton W, Arreaza L and Thibaud R. 2004. Inhibitory effect of flowering and early fruit growth on leaf photosynthesis in mango. Tree Physiology 24(3): 387-399. http://dx.doi.org/10.1093/treephys/24.4.38

Participações em memórias de congressos, seminários, simpósios: García M. 1998. La ingeniería geotécnica y la protección del medio ambiente. p. 65-94. Em: Memorias. IX Congreso Colombiano de la Ciencia del Suelo. Sociedad Colombiana de la Ciencia del Suelo. Santa Fé de Bogotá.

Teses, trabalhos de formatura. Gómez C. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 78 p.

Abril G. 2002. Biogeografía y descripción de las especies del género *Collaria* sp. en seis zonas lecheras del Departamento de Antioquia, Trabalho de formatura. Facultad de Ciencias Agropecuarias. Universidad Nacional de Colombia. Medellín. 49 p.

Citação de citação. Magalhaes LM e da Cruz AJ. 1979. Fenologia do pau-rosa (*Aniba duckei* Kostermans), Lauraceae, em floresta primária na Amazônia Central. Acta Amazónica. 9(2): 227-232. Citado por: Gómez CP. 2004. Autoecología del mortiño (*Vaccinium meriodinale* Swartz Ericaceae). Tese Mestrado em Bosques e Conservação Ambiental. Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia. Medellín. 46 p.

Suplemento de revista. Silva AM y Carrillo NN. 2004. El manglar de piruja, Golfito, Costa Rica: un modelo para su manejo. Revista de Biología Tropical 52, Supl. 2: 195-201.

Para citas de internet: Autor(es), ano. Título do artigo. Em: Nome(s) da publicação eletrônica, da página web, portal ou página e sua URL, páginas consultadas (pp.#) ou páginas totais (# p.); data de consulta. Exemplo: Arafat Y. 1996. Siembra de olivos en el desierto palestino. Em: Agricultura Tropical, http://agrotropical. edunet.es. 25 p.; consulta: novembro 2003.

ÉTICA EN LA PUBLICACIÓN CIENTÍFICA Y ACUERDO SOBRE POSIBLES MALAS PRÁCTICAS

La revista Facultad Nacional de Agronomía espera y verificará que los autores, revisores, editores y en general la comunidad académica y científica involucrada en nuestro proceso editorial, sigan estrictamente las normas éticas internacionales requeridas en el proceso de edición.

La revista Facultad Nacional de Agronomía sigue las normas éticas presentes en el COPE Best Practice Guidelines for Journal Editors y por el International Standars for Editors and Authors publicado por Committee on Publication Ethics.

Los autores deben evitar incurrir al plagio de la información. La revista define los siguientes lineamientos, criterios y recomendaciones sobre la ética en la publicación científica:

1. Criterios generales[1]

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

1.2.Declaraciones fraudulentas o deliberadamente inexactas constituyen un comportamiento poco ético.

1.3. Si el estudio incluye productos químicos, procedimientos o equipos que tienen cualquier riesgo inusual inherente a su uso, el autor debe identificar claramente estos en el artículo.

1.4. Si el estudio implica el uso de animales o de seres humanos, el autor debe asegurarse que el artículo contenga una declaración que haga explícito que se realizaron todos los procedimientos de conformidad con las leyes y directrices institucionales.

1.5. Se deben respetar los derechos de privacidad de los seres humanos.

2. Autoría[2]

Criterios:

2.1. Un "autor" es la persona que ha hecho una contribución intelectual significativa al artículo, por lo tanto, todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que los reúnan deben ser mencionados de forma explícita.

2.2. Se deben cumplir colectivamente tres criterios básicos para ser reconocido como autor:

a) Contribución sustancial a la concepción y diseño, adquisición de datos, análisis e interpretación del estudio.

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c) Aprobación de la versión final.

2.3. El orden de la autoría debe ser una decisión conjunta de los coautores.

2.4. Las personas que participen en un estudio pero que no se ajusten a los criterios de autoría deben aparecer como "Colaboradores" o "Personas reconocidas".

2.5. Hay tres tipos de autorías que se consideran inaceptables: autores "fantasma", que contribuyen sustancialmente pero no son reconocidos (a menudo pagados por promotores comerciales); autores "invitados", que no hacen ninguna contribución discernible pero se nombran para aumentar las posibilidades de publicación; y autorías "honorarias", que se basan únicamente en una afiliación tenue con un estudio.

Recomendaciones:

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

2.7. No se debe mentir sobre la participación de una persona en la investigación o publicación, si su contribución se considerada "sustancial" se justifica la autoría, bien sea como coautor o colaborador.

2.8. No se debe asignar una autoría sin contar con el consentimiento de la persona.

2.9. Todas las personas nombradas como autores deben reunir los requisitos de autoría, y todos aquellos que reúnan los requisitos deben aparecer como autores o contribuidores.

2.10. Algunos grupos colocan los autores por orden alfabético, a veces con una nota para explicar que todos los autores hicieron contribuciones iguales al estudio y la publicación.

3. Cambios en la autoría[3]

Criterios:

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

3.2. Las peticiones de añadir o eliminar un autor, o para reorganizar los nombres de los autores, deben ser enviados por el autor correspondiente del artículo aceptado, y deben incluir:

a) La razón por la cual debe ser añadido o eliminado, o los nombres de los autores reorganizado.

b) La confirmación por escrito (e-mail) de todos los autores que están de acuerdo con la adición, supresión o reorganización. En el caso de adición o eliminación de los autores,esto incluye la confirmación de que el autor sea añadido o eliminado.

4. Conflicto de intereses[4]

Criterios:

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

4.2. Los conflictos de intereses más evidentes son las relaciones financieras, como:

a) Directas: empleo, propiedad de acciones, becas, patentes.

b) Indirectas: honorarios, asesorías a organizaciones promotoras, la propiedad de fondos de inversión, testimonio experto pagado.

4.3. Los conflictos también pueden existir como resultado de relaciones personales, la competencia académica y la pasión intelectual. Por ejemplo, un investigador que tenga:

a) Algún tipo de interés personal en los resultados de la investigación.

b) Opiniones personales que están en conflicto directo con el tema que esté investigando.

Recomendaciones:

4.4. Revelar si se está en algún conflicto real o potencial de intereses que influya de forma inapropiada en los hallazgoso resultados del trabajo presentado, dentro de los tres (3) años de haber empezado el trabajo presentado que podría influir indebidamente (sesgo) el trabajo.

4.5. Revelar el papel de un promotor (o promotores) del estudio, si los hubiere, en el diseño del estudio, en la recopilación, análisis e interpretación de los datos, en la redacción del informe y en la decisión de presentar el documento para su publicación.

4.6. Los investigadores no deben entrar en acuerdos que interfieran con su acceso a todos los datos y su capacidad de analizarlos de forma independiente, y de preparar y publicar los manuscritos.

4.7. Al presentar un documento, se debe hacer una declaración (con el encabezamiento "Papel que ha tenido la fuente de financiación") en una sección separada del texto y colocarse antes de la sección "Referencias".

4.8. Algunos ejemplos de posibles conflictos de intereses que deben ser revelados, incluyen: empleo, consultoría, propiedad de acciones, honorarios, testimonio experto remunerado, las solicitudes de patentes / registros y subvenciones u otras financiaciones.

4.9. Todas las fuentes de apoyo financiero para el proyecto deben ser revelados.

4.10. Se debe describir el papel del patrocinador del estudio.

5. Publicación duplicada[5]

Criterios:

5.1. Los autores tienen la obligación de comprobar que su artículo sea basado en una investigación original (nunca publicada anteriormente). El envío o reenvío intencional de su trabajo para una publicación duplicada se considera un incumplimiento de la ética editorial.

5.2. Se produce una publicación duplicada o múltiple cuando dos o más artículos, sin hacerse referencias entre sí, comparten esencialmente las

mismas hipótesis, datos, puntos de discusión y/o conclusiones. Esto puede ocurrir en diferentes grados: Duplicación literal, duplicación parcial pero sustancial o incluso duplicación mediante parafraseo.
5.3. Uno de los principales motivos por los que la publicación duplicada de investigaciones originales se considera no ético es porque puede dar lugar a una "ponderación inadecuada o a un doble recuento involuntario" de los resultados de un estudio único, lo que distorsiona las pruebas disponibles.

Recomendaciones:

5.4. Los artículos enviados para su publicación deberán ser originales y no deberán haberse enviado a otra editorial. En el momento del envío, los autores deberán revelar los detalles de los artículos relacionados (también cuando estén en otro idioma), artículos similares en prensa y traducciones.
5.5. Aunque un artículo enviado esté siendo revisado y no conozca el estado, espere a que la editorial le diga algo antes de ponerse en contacto con otra revista, y sólo si la otra editorial no publicará el artículo.
5.6. Evite enviar un artículo previamente publicado a otra revista.
5.7. Evite enviar artículos que describan esencialmente la misma investigación a más de una revista.
5.8. Indique siempre los envíos anteriores (incluidas las presentaciones de reuniones y la inclusión de resultados en registros) que pudieran considerarse una publicación duplicada.
5.9. Evite escribir sobre su propia investigación en dos o más artículos desde diferentes ángulos o sobre diferentes aspectos de la investigación sin mencionar el artículo original.
5.10. Se considera manipulador crear varias publicaciones a raíz de la misma investigación.
5.11. Si desea enviar su artículo a una revista que se publica en un país diferente o en un idioma diferente, pregúntaselo a la editorial si se puede hacer esto.
5.12. En el momento del envío, indique todos los detalles de artículos relacionados en un idioma diferente y las traducciones existentes.

6. Reconocimiento de las fuentes

Criterios:

6.1. Los autores deben citar las publicaciones que han sido influyentes en la determinación de la naturaleza del trabajo presentado.
6.2. Información obtenida de forma privada, no debe ser usada sin explícito permiso escrito de la fuente.
6.3. La reutilización de las tablas y / o figuras requiere del permiso del autor y editor, y debe mencionarse de manera adecuada en la leyenda de la tabla o figura.
6.4. La información obtenida en el transcurso de servicios confidenciales, tales como manuscritos arbitrales o las solicitudes de subvención, no debe ser utilizada sin el permiso explícito y por escrito del autor de la obra involucrada en dichos servicios.

7. Fraude científico[6]

Criterios:

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

Recomendaciones:

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

8. Plagio[7]

Criterios:

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

Recomendaciones:

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

9. Fragmentación[8]

Criterios:

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

Recomendaciones:

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

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

10. Consentimiento informado

Criterios:

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

10.2. El consentimiento informado debe estar debidamente documentado.

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

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

11. Corrección de artículos publicados[9]

Criterio:

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

Referencias

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

Elsevier. «Autoría. Ethics in research & publication». Accedido 8 de agosto de 2014. http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

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[1] Elsevier, «Ethics. Conducting research», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#conducting-research.

[2] Elsevier, «Autoría. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0010/183394/ETHICS_ES_AUTH01a_updatedURL.pdf.

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

[4] Elsevier, «Conflicto de intereses. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0006/183399/ETHICS_ES_COI01a_updatedURL.pdf.

[5] Elsevier, «Envío simultáneo/múltiple, publicación duplicada. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0019/183403/ETHICS_ES_SSUB01a_updatedURL.pdf.

[6] Elsevier, «Fraude en investigación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0017/183401/ETHICS_ES_RF01a_updatedURL.pdf.

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

[8] Elsevier, «Fragmentación. Ethics in research & publication», accedido 8 de agosto de 2014, http://www.elsevier.com/__data/assets/pdf_file/0018/183402/ETHICS_ES_SS01a_updated updatedURL.pdf.

[9] Elsevier, «Ethics. Writing an article», accedido 8 de agosto de 2014, http://www.elsevier.com/journal-authors/ethics#writing-an-article.

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT

The journal Revista Facultad Nacional de Agronomia follows the COPE Code of Conduct and Best Practice Guidelines for Journal Editors and the International Standards For Editors and Authors, published by Committe on Publication Ethics.
The journal puts forth the following criteria and recommendations for ethical scientific publications:

1. General criteria[1]

1.1. Articles must contain sufficient details and references that allow the study to be replicable or refutable.
1.2. Fraudulent or deliberately inexact statements constitute unethical behavior.
1.3. If a study includes the use of chemical products, procedures, or equipment that presents an inherent risk, the author must state so in the article.
1.4. If the study involves the use of animals or human beings, the article must contain a clear statement that all of the procedures were carried out in strict compliance with laws and institutional directives.
1.5. The privacy of the human beings must be respected.

2. Authorship[2]

Criteria:
2.1. An "author" is a person that has made a significant intellectual contribution to an article; all of the individuals that are named as authors must fulfill the requirements for authorship and all of those individuals that do so must be explicitly named.
2.2. Three basic criteria must be met in order to be considered an author:
a) Substantial contribution to the study concept, design, and data collection, analysis and interpretation.
b) Revision of the intellectual content.
c) Approval of the final version.
2.3. The order of the author list must be a joint decision of the coauthors.
2.4. The individuals that participate in a study but that do not meet the criteria for authorship must be listed as an "Assistant" or "recognized person."
2.5. There are three types of unacceptable authorship: "ghost" authors, who make a substantial contribution but are not recognized (often paid by commercial promoters); "guest" authors, who do not make a discernable contribution but are named in order to increase the probability of publication; and "honorary" authors, who only have a tenuous connection to the study.
Recommendations:
2.6. Before starting the research, establish the function of each researcher and the manner in which they will be recognized.
2.7. It is not necessary to mention an individual's participation in a study or publication, but if their contribution is substantial, than authorship would be justified, either as an author or assistant.
2.8. Authorship cannot be bestowed on an individual without their consent.
2.9. All of the individuals that are named as authors must meet the requirements for authorship and all of those that meet the requirements must appear as authors or assistants.
2.10. Some groups list the authors alphabetically, sometimes with a notation that indicates that all of the authors contributed equally to the study and the publication.

3. Changes in the authorship[3]

Criteria:
3.1. Additions to, removals from, and reorganization of the author names in accepted articles must be noted.
3.2. Petitions to add to, remove from, or reorganize the authors must be sent by the corresponding author of the accepted articles and must include:
a) The reason for the addition, elimination, or reorganization.
b) A written statement (e-mail) from all of the authors that confirms their agreement with the addition, elimination, or reorganization. In the case of an addition or elimination, a confirmation is also required from the author to be added or removed.

4. Conflict of interest[4]

Criteria:
4.1. When a researcher or author has a financial/personal opinion or interest that could affect their objectivity or improperly influence their actions, there exists a possible conflict of interest. Conflicts can be actual or potential.
4.2. The most evident conflicts of interest are financial, such as:
a) Direct: employment, stocks, scholarships, patents.
b) Indirect: assistantship to promoting organizations, investment funds, paid expert testimony.
4.3. Conflicts can also arise from personal relationships, academic competition, and intellectual passion. For example, an author could have:
a) Some personal interest in the results of the research.
b) Personal opinions that are in direct conflict with the research topic.
Recommendations:
4.4. Disclose all conflicts of interest, actual or potential, that inappropriately influence the findings or results of a study, including any that arise within the three (3) years after the start of said study if they could unduly (bias) influence the study.
4.5. Disclose the role of any promoter (or promoters) in the study, if any, in the design, in the collection, analysis or interpretation of the data, in the document review, or in the decision to present the document for publication.
4.6. The researchers must not enter into agreements that interfere with their access to all of the data or with their ability to independently analyze the data or to prepare and publish the manuscript.
4.7. The document must contain a statement (with the heading "Role of the financial source") in a section that is separate from the text and before the References section.
4.8. Some examples of conflicts of interest that must be revealed include: employment, consulting, stocks, honorariums, paid expert testimony, patent requests or registration, and subsidies or other financing.
4.9. All of the sources of financial support for the project must be revealed.
4.10. The role of any study sponsors must be described.

5. Duplicate publication[5]

Criteria:
5.1. Authors have the obligation of proving that their article is based on original research (never before published). The intentional submission or resubmission of a manuscript for duplicate publication is considered a breach of editorial ethics.
5.2. A duplication publication, or multiple publication, results when two or more articles, without any reference to each other, essentially share the same hypothesis, data, discussion points, and/or conclusions. This can occur to different degrees: literal duplication, partial but substantial duplication or paraphrasal duplication.
5.3. One of the main reasons that duplicate publications are considered unethical is that they can result in the "inappropriate weighting or unwitting double counting" of results from just one study, which distorts the available evidence.
Recommendations:
5.4. Articles sent for publication must be original and not sent to other editors. When sent, the authors must reveal the details of related articles (even when in another language) and similar articles being printed or translated.

5.5. Even though a submitted article is being reviewed and the final decision is not known, wait to receive notification from the editors before contacting other journals and then only do so if the editors decline to publish the article.
5.6. Avoid submitting a previously published article to another journal.
5.7. Avoid submitting articles that essentially describe the same research to more than one journal.
5.8. Always indicate previous submissions (including presentations and recorded results) that could be considered duplicate results.
5.9. Avoid writing about your research in two or more articles from different angles or on different aspects of the research without mentioning the original article.
5.10. Creating various publications based on the same research is considered a type of manipulation.
5.11. If an author wishes to send an article to a journal that is published in a different country or a different language, ask for permission from the editors first.
5.12. When submitting an article, indicate all of the details of the article that were presented in a different language along with the relevant translations.

6. Acknowledging sources

Criteria:

6.1. Authors must cite the publications that had an influence on the determination of the nature of the offered study.
6.2. Privately obtained information cannot be used without the express written consent of the source.
6.3. Republishing tables or figures requires the permission of the author or editor, who must be appropriately cited in the table or figure legend.
6.4. Information obtained through confidential services, such as arbitration articles or subsidy applications, cannot be used without the express written consent of the author of the work involved in said services.

7. Scientific fraud[6]

Criteria:

7.1. Fraud in scientific publications refers to the presentation of false data or conclusions that were not obtained through a rigorous research process.
7.2. The following types of fraud exist for the publication of research results:
a) Fabricating data. Inventing research data and results for later dissemination.
b) Falsification of data. The manipulation of research material, images, data, equipment or processes. Falsification includes the modification or omission of data or results in such a way that the research is not represented in a precise manner. A person may falsify data in order to obtain the desired final results of a study.

Recommendations:

7.3. Before submitting an article, carefully read the editorial and data policies of the journal.
7.4. Never modify, change or omit data intentionally. This includes research material, processes, equipment, tables, citations, and bibliographical references.
7.5. Fabricating and falsifying data constitute grave misconduct because both result in scientific publications that do not precisely reflect the actual observations.
7.6. Authors must appropriately manage the data that supports the research, taking special care in the compilation, production, preservation, analysis and presentation of the data.
7.7. Maintain precise records of the raw data, which must be assessable in case the editors request them after publication of the article.

8. Plagiarism[7]

Criteria:

8.1. Plagiarism is one of the more common types of misconduct in publications; it occurs when an author passes the work of others off as their own without permission, citations, or acknowledgment. Plagiarism can occur in different forms, from literally copying to paraphrasing the work of another person, including data, ideas, concepts, paragraphs, and phrases.
8.2. Plagiarism has different degrees of severity; for example:
a) The quantity of work taken from another person (various lines, paragraphs, pages, or the entire article).
b) What is copied (results, methods, or introduction section).
8.3. Plagiarism, in all of its forms, constitutes unethical behavior and is unacceptable.
8.4. Literal copying is acceptable if the source is indicated and the text is placed in quotation marks.

Recommendations:

8.5. Always remember that it is vital to recognize the work of others (including the work of your assistants or your previous studies).
8.6. Do not reproduce the work of others word for word, in totality or partially, without the permission and recognition of the original source.
8.7. Maintain a record of the sources that are used in the research and where they are used in the article.
8.8. Be sure to accurately acknowledge and cite the original source in your article.
8.9. Even when referencing the source, avoid using the work of others word for word unless it is placed in quotations.
8.10. Paraphrasing is only acceptable if the source is correctly indicated and the source's intended meaning is not changed.
8.11. Use quotations, and cite all of the content that is taken from a previously published source even when using your own words.

9. Fragmentation[8]

Criteria:

9.1.Fragmentation occurs when a large study is divided or segmented into two or more publications.
9.2. As a general rule, as long as the "fragments" of a divided study share the same hypothesis, populations, and methods, this not considered an acceptable practice.
9.3. The same "fragment" can never be published more than one time. Fragmentation can result in distortion of the literature, creating the mistaken belief in readers that the data presented in each fragment (i.e. journal article) are derived from different subject samplings. This not only distorts the "scientific database", but creates repetition that results in a loss of time for editors and evaluators that must work on each article separately. Furthermore, the cited author receives an unfair increase in their number of references.

Recommendations:

9.4. Avoid inappropriately dividing the data of one study into two or more articles.
9.5. When presenting your work, be transparent. Send copies of the manuscripts that are closely related to the manuscript in question, including published, recently submitted and accepted manuscripts.

10. Informed consent

Criteria:

10.1. Studies on patients and volunteers require the approval of the ethics committee.
10.2. The informed consent must be duly documented.
10.3. Permission and waivers must be obtained when an author wishes to include details of a case or other personal information or images of the patients or any other person.
10.4. Special care should be taken when obtaining the consent

of children (especially when a child has special needs or learning disabilities) when their head or face is displayed or when reference is made to the name of an individual or other personal data.

11. Correction of published articles[9]

Criterion:

When an author discovers a significant inexactitude or error in a published article, they must immediately notify the journal and cooperate in the correction process.

References

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