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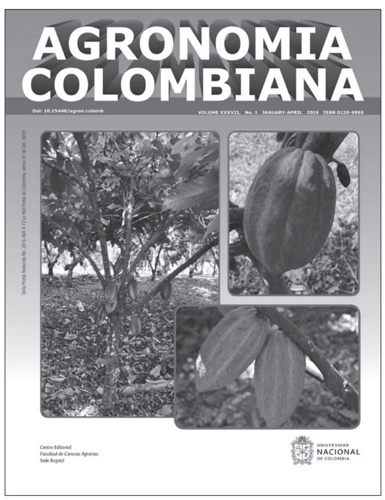

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Physicochemical characteristics of cacao (*Theobroma cacao* L.) soils in Colombia: Are they adequate to improve productivity?
Article on pages: 28-38

The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L.) production activities in Santander, Colombia
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Physiological and antioxidant responses of cape gooseberry (*Physalis peruviana* L.) seedlings to phosphorus deficiency

Respuestas fisiológicas y antioxidantes en plántulas de uchuva (*Physalis peruviana* L.) a la deficiencia de fósforo

Gabriel Roveda-Hoyos[1] and Liz Moreno-Fonseca[1*]

ABSTRACT

The main objective of present study was to understand the physiological effects of phosphorus (P) deficiency and the antioxidant response in cape gooseberry (*Physalis peruviana* L.) seedlings. Seedlings were grown in soil with five P levels: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1}. The plant growth, gas exchange, chlorophyll content, membrane integrity and the antioxidant response in cape gooseberry were evaluated. In the P0, P6, P12 treatments, the seedlings showed a reduction in total biomass, the number of leaves, leaf area, root length density, shoot/root ratio, photosynthesis, transpiration, stomatal conductance, and chlorophyll content, as well as an increase in the electrolyte leakage, the proline content and the activity of catalase and peroxidase compared with the P50 treatment. The P25 treatment was not different compared to P50 in terms of photosynthesis, chlorophyll content and total biomass after 30 d of treatment, the number of leaves and root length density at 90 d of treatment, and in electrolyte leakage and peroxidase activity at 60 and 90 d of treatment. Doses below 25 mg of P_2O_5 kg^{-1} cause P deficiency in cape gooseberry seedlings, inducing antioxidant and protection response mechanisms to cope with stress.

Key words: catalase, electrolyte leakage, mineral nutrition, proline, root length density.

RESUMEN

El presente estudio tuvo como objetivo comprender los efectos fisiológicos de la deficiencia de fósforo (P) y la respuesta antioxidante de las plántulas de uchuva (*Physalis peruviana* L.). Las plántulas se cultivaron en suelo con 5 niveles de P: 0 (P0), 6 (P6), 12 (P12), 25 (P25) y 50 (P50) mg de P_2O_5 kg^{-1}. Se evaluaron el crecimiento, el intercambio gaseoso, el contenido de clorofila, la integridad de la membrana y la respuesta antioxidante en las plántulas de uchuva. En los tratamientos P0, P6, P12, las plántulas mostraron una reducción en la biomasa total, el número de hojas, el área foliar, la densidad de la longitud de las raíces, la relación entre las raíces y la parte aérea, la fotosíntesis, la transpiración, la conductancia estomática y el contenido de clorofila, así como un incremento en la pérdida de electrolitos, el contenido de prolina y la actividad de la catalasa y la peroxidasa en comparación con el tratamiento con P50. El tratamiento con P25 no fue diferente con respecto a P50 en términos de fotosíntesis, contenido de clorofila y biomasa total después de 30 días de tratamiento, y en cuanto al número de hojas y la densidad de longitud de raíces a los 90 días de tratamiento y en la pérdida de electrolitos y actividad peroxidasa a 60 y 90 días de tratamiento. Dosis menores de 25 mg de P_2O_5 kg^{-1} causan deficiencia de P en las plántulas de la uchuva, induciendo la producción de antioxidantes y mecanismos de respuesta de protección para atenuar los efectos del estrés.

Palabras clave: catalasa, pérdida de electrolitos, nutrición mineral, prolina, densidad de longitud de raíces.

Introduction

Physalis peruviana L. is a plant of Andean origin cultivated in South America, from Venezuela to Chile. Nowadays, it is grown in other parts of the world, such as California, South Africa, India, New Zealand, Australia and Great Britain (Ramadan and Mörsel, 2003; El-Tohamy *et al.*, 2012). *P. peruviana* L. fruits are round, bright orange and have a pleasant taste related to their tropical origin (Fischer *et al.*, 2007). This species is very important due to its high nutritional value and potential health benefits, derived from the high content of ascorbic acid, vitamins and antioxidants (Puente *et al.*, 2011; Briones-Labarca *et al.*, 2013). The high prices paid for cape gooseberries in local and international markets have made this plant attractive to producers (Fischer *et al.*, 2007; Ramírez *et al.*, 2013).

One of the main limiting factors of agricultural production, especially in the tropics and subtropics, is the phosphorus (P) deficiency (Sánchez, 1976; Ramaekers *et al.*, 2010). This element has an important role in various processes, such as nucleic acid synthesis, energy production,

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photosynthesis, respiration, synthesis and stability of the membrane, enzyme activation and signaling (Vance *et al*., 2003; Hawkesford *et al*., 2012; Manschadi *et al*., 2014). It has been reported that P deficiency reduces the production of biomass in leaves, affecting plant growth (Kirschbaum and Tompkins, 1990; Plénet *et al*., 2000; De Groot *et al*., 2001; De Groot *et al.,* 2003; Chaudhary *et al*., 2008; Maathuis, 2009; Reich *et al*., 2009). A change in the root/shoot ratio due to an increase in the density of root hairs, an adaptive response of plants to P deficiency, has also been reported (Mollier and Pellerin, 1999; Hermans *et al*., 2006; Yao *et al*., 2007; Zhang *et al*., 2013).

The decrease in CO_2 assimilation due to P deficiency is mainly associated to a decline in the regeneration of Rubisco (Jacob and Lawlor, 1992; Rao and Terry, 1995; Campbell and Sage, 2006; Singh *et al*., 2013) and stomatal closure caused by the accumulation of CO_2 in intercellular spaces (Thomas *et al*., 2006). This effect has also been observed in corn (Tewari *et al*., 2004) and rice (Guo *et al*., 2012). Phosphorus deficiency decreases the chlorophyll content, thus affecting photosynthesis. The imbalance in photosynthesis phases generated by P deficiency can cause an increase of reactive oxygen species (ROS), which alter plant functions by damaging lipids, proteins, enzymes, nucleic acids and photosynthetic pigments. Moreover, an increase in free radical production has been reported in bean (*Phaseolus vulgaris* L.) roots in plants with phosphate deficiency (Malusa *et al*., 2002). ROS damage cell membranes, causing changes in permeability and resulting in electrolyte leakage (EL) and alterations in cellular metabolism (Blokhina *et al*., 2003).

In order to reduce oxidative damage, plants have developed different response mechanisms, especially the production of antioxidants and protective molecules (Cruz de Carvalho, 2008). P deficiency in maize (Tewari *et al*., 2004) and rice (Guo *et al*., 2012) has been observed to cause a significant increase in the activity of antioxidant enzymes, such as catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD), and an increase in the content of proline (Pro). In tomato plants, which are tolerant to low P availability, increased peroxidase activity and anthocyanin accumulation have been observed (Khavari-Nejad *et al*., 2009). Also, an increase in the proline content of roots and stems (Sarker and Karmoker, 2011) has been found in lentil plants (*Lens culinaris* Medik).

P. peruviana L. is grown from 1500 m to 3000 m a.s.l. in the Andes, often in volcanic soil with an acidic pH and low phosphorus availability due to the high binding capacity of phosphate anions in the soil solution (Fischer *et al*., 2007; Ramirez *et al*., 2013). These P limitations affect both the yield and quality of cape gooseberry plants (Garzón-Acosta *et al*., 2014). However, there have not been any studies that describe the effects of phosphorus deficiency on the physiology of the plant at different stages of development. The purpose of this research was to determine the response mechanisms of cape gooseberry (*P. peruviana* L.) seedlings in the early stages of development under conditions of phosphorus deficiency through the analysis of morphological, physiological and biochemical responses.

Materials and methods

Plant material and experimental design

Cape gooseberry (*P. peruviana* L.) seeds ecotype Colombia were germinated in peat moss in a growth chamber with a photosynthetic photon flux density (PPFD) of 350 µmol m^{-2} s^{-1}, 70% relative humidity, with 12 h of light at 22°C and 12 h of darkness at 10°C. Twenty days after germination, the seedlings were transplanted to 50-cell trays with an inert substrate (2:1:1 mixture of river sand, quartz sand and fine granite). A 0.8 strength Hoagland solution and the designated P doses were applied. The seedlings grew in a greenhouse with a PPFD range between 450-900 µmol m^{-2} s^{-1} PAR (photosynthetic active radiation), 70% relative humidity, 12 h of light at 25°C and 12 h of darkness at 8°C. A completely randomized block design with five treatments, four replications and an experimental unit of eight plants was used. The treatments had five levels of P_2O_5 kg^{-1} in the substrate: 0 mg P_2O_5 kg^{-1} (P0), 6 mg P_2O_5 kg^{-1} (P6), 12 mg P_2O_5 kg^{-1} (P12), 25 mg P_2O_5 kg^{-1} (P25) and 50 mg P_2O_5 kg^{-1} (P50). The variables were measured at 15 d of treatment (dt), 30 dt, 60 dt and 90 dt.

Growth parameters

The plants were individually divided into roots, stems and leaves and the number of leaves (NL) and the leaf area (LA) were measured using a portable area meter (CI-202, CID Inc, USA). Then, the plant material was dried at 70°C until a constant weight was achieved. The root/shoot ratio was determined using these data and the root length density (RLD) was determined according to Newman and Ritz (1986).

Gas exchange

The photosynthetic rate (A), stomatal conductance (gs) and transpiration (E) were registered at 90 dt using a photosynthesis measurement system (LCpro-SD, Portable BioScientific, UK). The measurements were taken from the fourth apical leaf on six plants per treatment, between 9:00

AM and 11:00 AM, with a CO_2 concentration of 360 µl L^{-1} and a PPDF of 900 µmol m^{-2} s^{-1}.

Chlorophyll content and leaf temperature

The pigments were extracted according to Cubas *et al.* (2008). The apical expanded leaves from six plants per treatment where homogenized in 80% acetone. The absorbance was determined at an optical density (OD) of 663 nm and 647 nm. The leaf temperature (LT) was determined with an infrared thermometer (HD550, Extech®, USA) on the fourth apical leaf.

Membrane permeability

The membranes permeability was measured by electrolyte leakage (EL) according to Rodríguez *et al.* (2005). Eight 2.5 mm diameter leaf discs were placed in Falcon tubes with 2 ml of deionized water at 24°C. The electric conductivity (EC) was determined with an electrical conductivity meter (HI 9835 HANA, USA) at 24 h. The EC values are expressed as a percentage with respect to the highest value using the following equation:

$$PE = (EC1*EC2-1)*100 \quad (1)$$

where: PE = % of lost electrolytes, EC1 = electric conductively at 24 h, and EC2 = electric conductivity after heating to 80°C. The measurements were carried out on the fourth apical leaf.

Antioxidant enzymes, proline and protein content

A 0.2 g powdered leaf sample was treated with polyvinylpyrrolidone 40 (22% w/w) and with 1.5 ml cold 80% (v/v) acetone and centrifuged (8000xg for 30 min, 4°C) to eliminate pigments, according to Lichtenthaler (1987). Afterwards, samples were extracted in 3 ml of 110 mM sodium phosphate buffer (pH 7.2) containing 3.82% (w/v) polyvinyl pyrrolidone 40. The homogenate was centrifuged (6000 x g, 1 h, 4°C) and the supernatant was used for the subsequent enzyme assays. The CAT activity was determined by the method of reduction of potassium permanganate ($KMnO_4$) with hydrogen peroxide in sulfuric acid (Ulrich, 1974). The POD activity was estimated by the absorbance change at 436 nm caused by the oxidation of guaiacol according to Kireyko *et al.* (2006). The proline level was determined according to Bates *et al.* (1973) based on the proline-ninhydrin reaction. For the proline (Pro) determination, a 1:1:1 solution of proline, ninhydrin and glacial acetic was incubated at 97°C for 1 h. The reaction was stopped by placing the vials in an ice bath; the chromophore was extracted with toluene and its absorbance was measured at 520 nm. The soluble proteins were measured based on the method of Bradford (1976), using bovine serum albumin as a reference.

Data analysis

An analysis of variance (ANOVA) was carried out to determine the effect of the treatments in the analyzed variables. The comparison of the means was done with a Tukey multiple range test (P<0.05).

Results and discussion

Growth parameters

The seedling dry weight decreased by reducing the concentration of P in the nutrient solution (Fig. 1A). This decrease was significant between P0 and P6 and the other treatments. The highest values for the dry weight were observed in P50 at 30 dt (0.60 g), 60 dt (4.63 g) and 90 dt (18.13 g) and the lowest values were observed in P0 at 30 dt (0.07 g) and 60 dt (0.08 g); the P0 plants died before 90 dt. Although there were differences between P12, P25 and P50 at 90 dt, the values were closer to the values of 30 dt and 60 dt. The assimilate partitioning in P0 and P6 was higher towards the root, compared to the other treatments at 30 dt and 60 dt (P0: 0.038 g, 0.047 g and P6: 0.043 g, 0.064 g, respectively). At 90 dt, there were differences in the partition of assimilates to the root between P12 and P25 as compared to P50, where fewer assimilates were partitioned to roots (Fig. 1B).

The root/shoot ratio was higher at 30 dt and 60 dt for P0 (1.1; 1.5; nd) and P6 (1.0; 0.8; 0.7) compared to the others treatments. P12 and P25 did not show differences in the root/shoot ratio between each other, but were higher than P50 (Tab. 1). The NL, LA and RLD progressively decreased with a reduction in the concentration of phosphorous in the nutrient solution (Tab. 1). At 90 dt, no differences were observed in the NL and RLD between P25 and P50, but the LA was lower in P25 (168.3 cm^2) than in P50 (196 cm^2). Phosphorus is an essential element for the growth and development of plants; however, there are differences in requirements due to the efficient use some plants make of this element (Kirschbaum and Tompkins, 1990; Nielsen *et al.*, 2001; Chaudhary *et al.*, 2008; Maathuis, 2009; Reich *et al.*, 2009). In the cape gooseberry seedlings, there was a decrease in the dry weight, number of leaves and leaf area as a result of a decrease in the dose of phosphorus; similar data have been reported for different species (Radin and Eidenbock, 1984; Guo *et al.*, 2012). The seedlings in the P0 treatment, without phosphorus, achieved little growth, possibly by using seed reserves, and did not survive beyond 60 dt. In addition to the reduction of total biomass

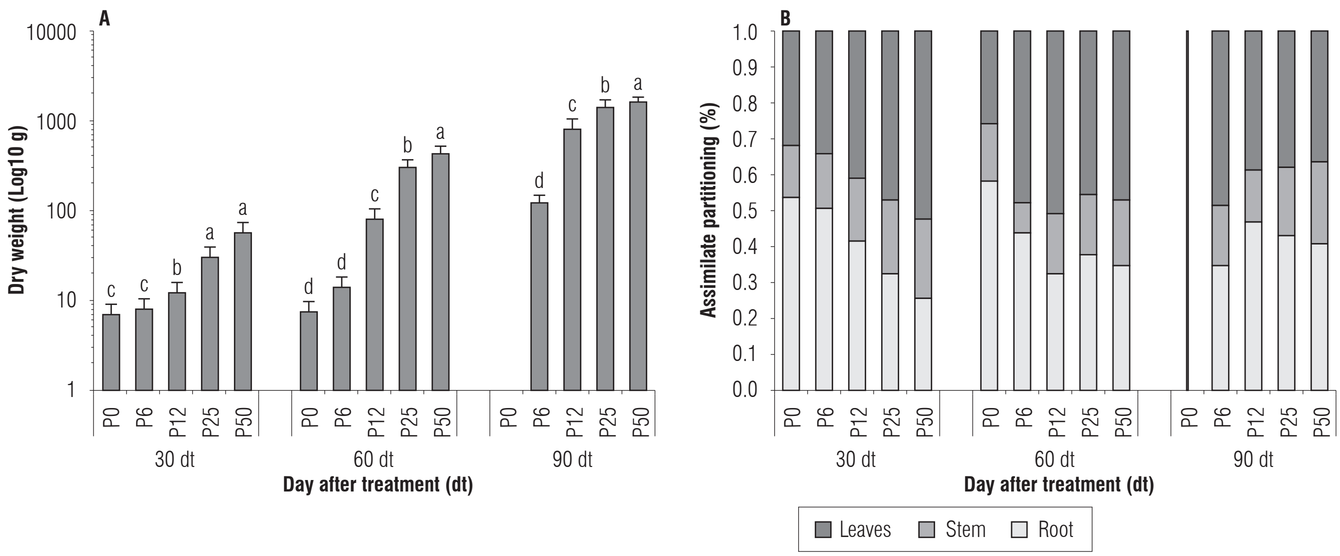

FIGURE 1. Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on the seedling dry weight, logarithmically transformed data (A) and assimilate partitioning (B) of the cape gooseberry seedlings (*Physalis peruviana* L.) at 30, 60 and 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test.

in plants, one of the indicators of phosphorus deficiency is an increase in the partitioning of photoassimilates to roots (Mollier and Pellerin, 1999; Hermans *et al*., 2006; Yao *et al*., 2007; Zhang *et al*., 2013). It was observed that all of the treatments, except P50, had a higher proportion of biomass in the roots and a higher root/shoot ratio. This response has been described for many plants suffering P deficiency as a strategy to get through the depleted zone in the substrate and obtain more P (Mollier and Pellerin, 1999; Hermans *et al*., 2006; Yao *et al*., 2007). These data suggest that the cape gooseberry seedlings in P6, P12 and P25 had a P deficiency. Likewise, the decrease in the difference in the dry weight of the seedlings at 90 dt between P12, P25 and P50 suggests that the seedlings changed their metabolism to increase the efficiency of P use, as has been reported in many plants (Nakamura, 2013).

Gas exchange and leaf temperature

The P25 and P50 treatments had higher A values (10 μmol $m^{-2}s^{-1}$; 10.54 μmol $m^{-2}s^{-1}$) as compared to P12 and P6 (2.76 μmol $m^{-2}s^{-1}$; 1.67 μmol $m^{-2}s^{-1}$) (Fig. 2A). E behaved similarly to A, with differences between P6 (0.26 mmol $m^{-2}s^{-1}$) and P50 (2.81 mmol $m^{-2}s^{-1}$) (Fig. 2B). P25 had the highest gs value (0.202 mmol $m^{-2}s^{-1}$) and P6 and P12 had lower values (0.025 mmol $m^{-2}s^{-1}$; 0.100 mmol $m^{-2}s^{-1}$) (Fig. 2C). The leaf temperature was higher in P0 (18.1°C) and P12 (16.1°C) compared to P25 (16.1°C) and P50 (14.8°C) (Fig. 2D). The temperature increased between 3.3°C and 3.9°C between the treatments with deficient P (P0 and P6) and the treatment with sufficient P (P50). Plant biomass production

TABLE 1. Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on the Number of Leaves per plant (NL), Leaf area (LA), Root Length Density (RLD) and Root Shoot ratio (Root/Shoot) in cape gooseberry (*Physalis peruviana* L.) seedlings at 30, 60 and 90 d after treatment (dt). The data shown are the averages of sixteen replicates. Pi, Phosphorous levels; nd, data not available.

dt	Pi	NL	LA	RLD
	(mg kg^{-1})		(cm^2)	(mm^3 cm^{-3})
30	P0	1.0 d	0.9 c	nd
30	P6	1.1 d	1.0 c	nd
30	P12	1.5 c	1.9 c	nd
30	P25	2.9 b	6.8 b	nd
30	P50	3.6 a	12.3 a	nd
	ANOVA	**	**	
60	P0	1.1 e	0.9 d	0.3 d
60	P6	2.3 d	2.0 d	0.7 d
60	P12	4.3 c	15.7 c	2.9 c
60	P25	5.9 b	43.9 b	12.2 b
60	P50	7.2 a	61.0 a	18.3 a
	ANOVA	**	**	**
90	P0	nd	nd	nd
90	P6	5.0 c	17.3 d	12.9 c
90	P12	7.6 b	94.3 c	51.5 b
90	P25	10.7 a	168.3 b	64.6 a
90	P50	11.1 a	196.0 a	70.5 a
	ANOVA	**	**	**

Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test. Comparisons among treatments were analyzed by one-way ANOVA. Statistical significance for $P<0.05$ (*), $P<0.01$ (**).

depends directly on net photosynthesis, which is related to the plant ability to intercept light and the efficiency of photosynthetic metabolism (Singh *et al*., 2013). Large decreases in A and gs were observed in P6 and P12, as compared to P50, possibly due to the decreased synthesis of ATP in the photosynthesis phase, which causes a limitation in the regeneration of the CO_2 acceptor ribulose-1,5-bisphosphate in the Calvin cycle. This slows carboxylation and increases the intercellular concentration of CO_2, which causes stomatal closure (Singh *et al*., 2013). Similar results have been reported in other species at P deficit conditions, such as Arabidopsis, sorghum, corn, beans and cotton (Radin and Eidenbock, 1984; Barrett and Gifford, 1995; Abel *et al*., 2002; Tewari *et al*., 2004; Yao *et al*., 2007; Chaudhary *et al*., 2008; Singh *et al*., 2013). Similarly, the decrease in gs caused by an imbalance in the two phases of photosynthesis produced a reduction in E and an increase in LT due to a decrease in energy dissipation. Although the seedlings in P25 had a higher root/shoot ratio than P50 and a decrease in dry weight accumulation, which indicates a deficiency of P, they did not show a decrease in A. This suggests that these plants exhibited a slight deficiency that affected cell division, but not the function of the photosynthetic apparatus.

Chlorophyll content

The chlorophyll content (Chl) decreased in P6 (1343 μg g^{-1} DW) and P12 (1330 μg g^{-1} DW) as compared to P25 (1615 μg g^{-1} DW) and P50 (1568 μg g^{-1} DW). The reduction in Chl in P6 compared to P50 was 14.3% (Fig. 3). A decrease in chlorophyll content due to P deficiency has been reported in plants such as maize (Tewari *et al*., 2004). In the case of Solanaceae, such as tomatoes, a severe reduction has been reported in the chlorophyll content with combined N and P deficiency (Khavari-Nejad *et al*., 2009). The reduction in the chlorophyll content can also be related to the degradation of these molecules by ROS (Misson *et al*., 2005). The decrease in the A may also have been related to the decrease in the chlorophyll content observed in P6 and P12.

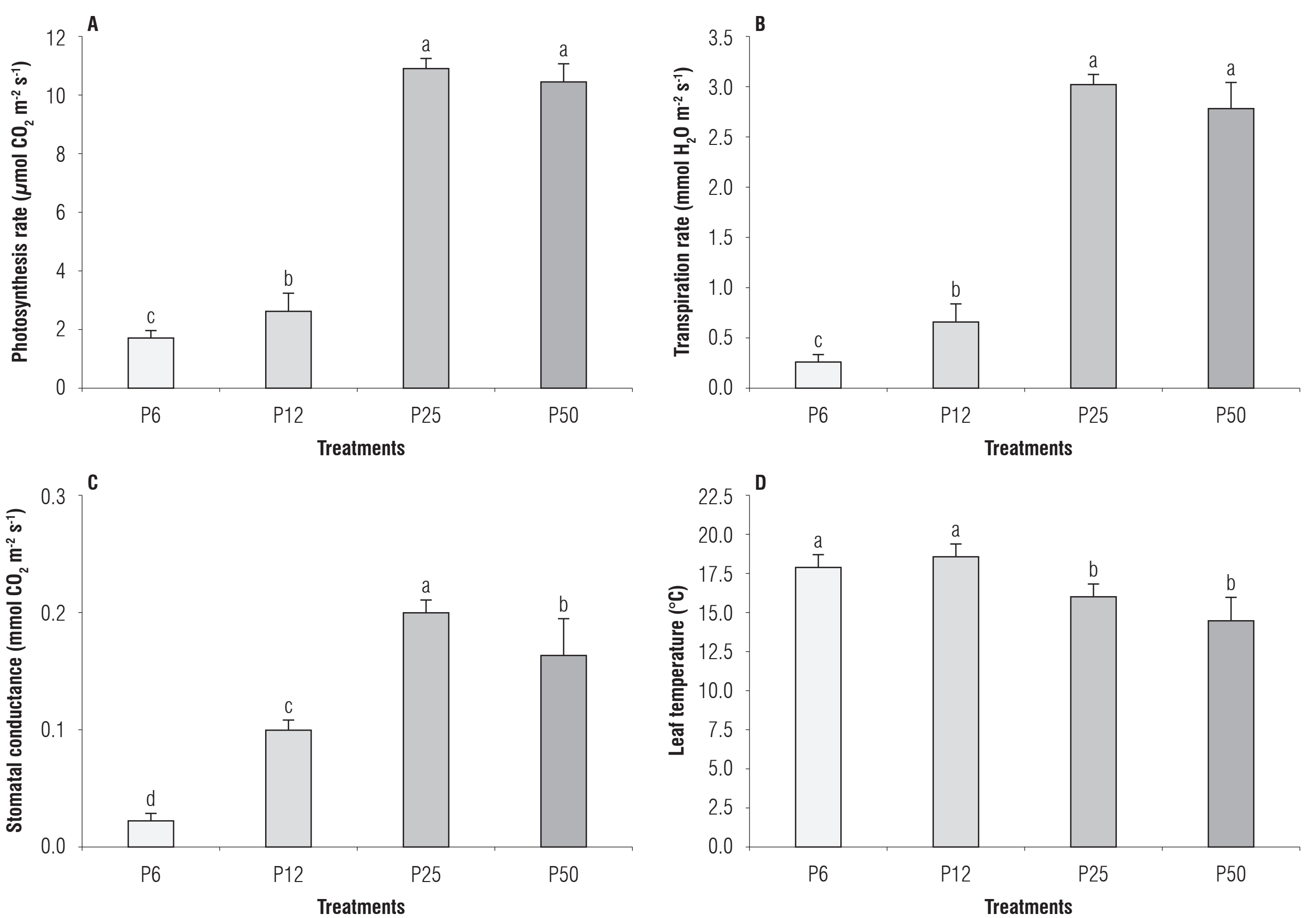

FIGURE 2. Effect of four phosphorous levels in the soil: 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on the gas exchange. A, photosynthesis rate (A), B, transpiration rate (E), C, stomatal conductance (gs) and D, leaf temperature (LT) in cape gooseberry (*Physalis peruviana* L.) seedlings at 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test.

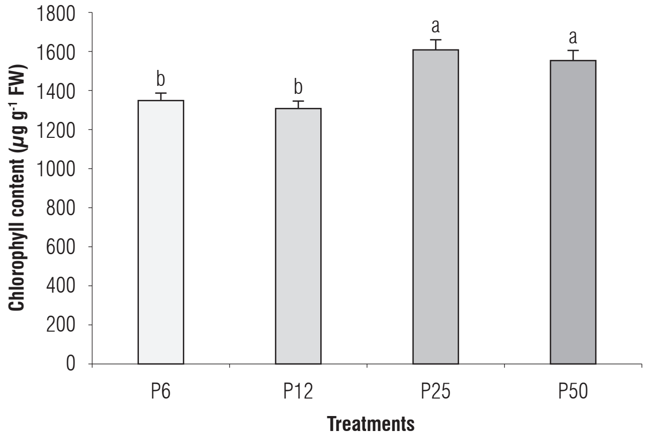

FIGURE 3. Effect of four phosphorous levels in the soil: 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on the chlorophyll content in cape gooseberry (*Physalis peruviana* L.) seedlings at 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test.

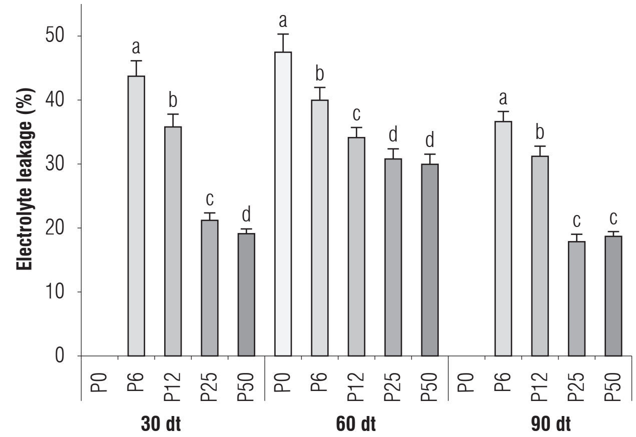

FIGURE 4. Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on electrolyte leakage in cape gooseberry (*Physalis peruviana* L.) seedlings at 30, 60 and 90 d of treatment (dt). The data shown are the averages of sixteen replicates, with the standard deviations indicated by the vertical bars. Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test.

Membrane permeability

The P0, P6 and P12 treatments had higher percentages of EL (Fig. 4). The EL for P6 at 30 dt, 60 dt and 90 dt was 44%, 40% and 37%, respectively, while for P50 it was 19%, 30% and 19%, respectively (Fig. 4). The P25 and P50 treatments showed no significant differences between each other at 60 and 90 dt. Due to the imbalance in the two phases of photosynthesis caused by ATP deficiency, there is an increase in ROS production, which caused damage to macromolecules and structures as membranes (Misson *et al.*, 2005). Here, there was an increase in EL in P0, P6 and P12, suggesting that P deficiency causes damage to membranes, affecting the permeability degree, probably due to lipid peroxidation induced by ROS.

Antioxidant enzymes, proline and protein content

The activity of the antioxidant enzyme CAT was determined in the leaves at 15 dt, 30 dt, 60 dt and 90 dt (Tab. 2). The CAT activity was higher for all of the treatments at 15 dt, when the activity in P0 (791 UA g^{-1} FW) was approximately 3.3 times higher than P50 (238 UA g^{-1} FW). The CAT activity gradually decreased over time in all of the treatments and, at 90 dt, presented lower values. Throughout the experiment, P50 had the lowest values of CAT activity (106.2 UA g^{-1} FW to 238.3 UA g^{-1} FW) (Tab. 2). The POD activity was low in all of the treatments, but had higher values in P6 (1.4 mg POD g^{-1} FW) and P12 (1.38 g^{-1} FW) at 15 dt. The POD activity gradually reduced until 90 dt, when the lowest value was observed for all treatments.

The contents of Pro throughout the experiment were higher in P0, P6 and P12 (Tab. 2). At 60 dt, P0 showed the highest value for the Pro content (771.7 mg g^{-1} FW), just before the plants died. At 90 dt, the Pro content in all of the treatments decreased between 101.8 mg g^{-1} FW (P6) and 29.4 mg g^{-1} FW (P50). The protein content was higher in all of the treatments at 90 dt, when the Pro content was lower (Tab. 2), with values between 2.5 mg g^{-1} FW (P25) and 2.7 mg g^{-1} FW (P6, P12). Higher antioxidant enzyme activity, such as peroxidase (POD) and superoxide dismutase (SOD), has been reported in rice seedlings (Guo *et al.*, 2012) and also in maize, with increase in other enzymes as CAT and ascorbate peroxidase (APX) under P deficiency (Tewari *et al.*, 2004). Additionally, in beans, P deficiency increases CAT and POD activities (Juszczuk *et al.*, 2001). In cape gooseberry seedlings, there was an increased CAT activity for the P0, P6, P12 and P25 treatments at 15 dt (Tab. 2). The increase was greater for the treatments with lower doses of P, showing that the expression of this enzyme is an early response in cape gooseberry seedlings to a deficiency of this element and may be related to the observed decrease in EL. Similarly, POD enzyme activity was low, but was higher in treatments with lower doses of P. It has also been reported that P deficiency can increase the Pro content in roots and stems in lentil plants (Sarker *et al.*, 2011) and in leaves and roots in beans that are deficient in P (Juszczuk *et al.*, 2001). The observed Pro increase in P0, P6 and P12, which was higher at 30 dt and 60 dt, could be another response of cape gooseberry seedlings to reduce ROS caused by an imbalance in photosynthesis (Hare *et*

TABLE 2. Effect of five phosphorous levels in the soil: 0 (P0), 6 (P6), 12 (P12), 25 (P25) and 50 (P50) mg of P_2O_5 kg^{-1} on the content of Catalase (CAT), Peroxidase (POD), Proline (Pro) and total protein in cape gooseberry seedlings (*Physalis peruviana* L.) at 15 dt, 30 dt, 60 dt and 90 dt. Pi, Phosphorous level; FW, fresh weight; nd: data not available.

dt	Pi	CAT	POD	Pro	Total Protein
	(mg kg^{-1})	(UA g^{-1} FW)	(UA g^{-1} FW)	(mg g^{-1} FW)	(mg g^{-1} FW)
15	P0	791.0 a	1.0 b	43.0 c	1.6 a
15	P6	620.4 b	1.4 a	139.4 b	1.7 a
15	P12	546.8 c	1.4 a	178.8 a	1.5 c
15	P25	336.5 d	0.3 c	24.0 d	1.2 d
15	P50	238.3 d	0.2 c	16.4 e	1.3 c
	ANOVA	**	**	**	**
30	P0	392.4 a	0.7 a	94.0 c	0.1 d
30	P6	379.8 a	0.9 a	273.0 b	0.6 c
30	P12	344.7 a	1.1 a	332.1 a	0.6 c
30	P25	231.0 b	0.2 b	44.6 d	1.1 b
30	P50	163.7 c	0.2 c	30.4 e	1.3 a
	ANOVA	**	**	**	**
60	P0	420.8 a	0.6 b	771.7 a	0.2 e
60	P6	365.0 b	0.6 b	282.8 b	0.8 d
60	P12	339.5 b	0.6 b	220.2 d	0.8 c
60	P25	267.2 c	0.1 a	166.2 d	1.5 b
60	P50	134.9 d	0.1 a	161.2 d	1.7 b
	ANOVA	**	**	**	**
90	P0	nd	nd	nd	nd
90	P6	313.9 a	0.1 a	101.8 a	2.7 b
90	P12	310.0 a	0.1 a	97.2 a	2.7 a
90	P25	148.0 b	0.1 a	64.2 b	2.5 d
90	P50	106.2 c	0.1 a	29.4 a	2.6 c
	ANOVA	**		**	**

Means denoted by the same letter do not significantly differ at $P<0.05$ according to the Tukey test. Comparisons among treatments were analyzed by one-way ANOVA. Statistical significance at $P<0.05$ (*), $P<0.01$ (**).

al., 1998). It has been reported that low molecular weight metabolites, such as Pro, are efficient at detoxifying the ˙OH radical, and increased Pro synthesis has been reported in response to different abiotic stresses or by combining phosphorus deficiency simultaneously with other stresses, such as drought stress (Al-Karaki *et al*., 1996) or salinity stress (Zribi *et al*., 2015). Pro accumulation has also been reported in response to stress nutrients such as Al and Ca in beans (Yang and Chen, 2001), or Al and P (Ismail, 2005; Guo *et al*., 2012) and Fe (Arias-Baldrich *et al*., 2015), but not under conditions of phosphorus deficiency alone. The observed Pro accumulation may have been a stress response to P deficiency to protect macromolecules and structures from damage caused by ROS, due to its function as a compatible osmolyte (Schobert and Tschesche, 1978). The increase in the defense and protective mechanisms in cape gooseberry seedlings was probably due to metabolic adjustment, which aims at reducing the effect of stress under moderate P deficiency, as noted here. The development of mechanisms such as increased antioxidant capacity and the synthesis of osmolytes has an energy cost that plants probably cannot assume with a severe phosphorus deficiency, as in P0 treatment, because the limitation imposed by deficiency is too high. Furthermore, the metabolic adjustment induced by P deficiency includes other mechanisms, such as the use of PPi-dependent enzymes in the glycolysis pathway and the replacement of membrane phospholipids with sulfolipids; plants have developed these mechanisms since P is a limiting element on the planet (Nakamura, 2013). These stress defense mechanisms, described here for the first time for *P. peruviana* L., may explain the acclimatization capability observed in cape gooseberry seedlings under moderate P deficiency.

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Effect of pollen doses on fruit formation and oil production in two hybrid palm genotypes (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)

Efecto de dosis de polen en la formación de frutos y producción de aceite en dos genotipos híbridos de palma (*Elaeis oleifera* H.B.K. Cortes x *Elaeis guineensis* Jacq.)

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ABSTRACT

OxG oil palm hybrids (*Elaeis oleifera* x *Elaeis guineensis*) produce a small number of male inflorescences, with an irregular, slow anthesis phase and low-viability pollen that affect natural pollination, making the agronomic practice of assisted pollination necessary for plantations in order to guarantee the formation of the majority of bunch fruits. In the hybrids "Coarí x La Mé" and "(Sinú x Coarí) x La Mé", the influence of several doses of pollen (0, 0.01, 0.05, 0.1, 0.15 g/inflorescence) on bunch weight, normal and parthenocarpic fruits, fruit set and oil/bunch potential was studied. A completely randomized experimental design was used with four replicates and nine inflorescences/replicate. The bunch weight was higher with the 0.05 g/inflorescence dose and lower with the natural pollination. The greatest fruit set was obtained with the dose of 0.1 g/inflorescence, and the oil/bunch percentage was higher with 0.05 g/inflorescence and lower with 0.01 g/inflorescence. The results confirm the need for assisted pollination in these new hybrid materials.

Key words: oil palm, physiology, pollination, fruit set, oil potential.

RESUMEN

Los híbridos OxG de palma de aceite (*Elaeis oleifera* x *Elaeis guineensis*) producen pocas inflorescencias masculinas con fase de antesis irregular lenta y polen de baja viabilidad afectando su polinización natural, lo cual hace necesaria la práctica agronómica de polinización asistida de las plantaciones para favorecer la formación de la mayoría de frutos del racimo. En los híbridos "Coarí x La Mé" y "(Sinú x Coarí) x La Mé" se estudió la influencia de varias dosis de polen (0, 0.01, 0.05, 0.1, 0.15 g/inflorescencia) sobre el peso del racimo, frutos normales y partenocárpicos, cuajado de frutos y potencial de aceite/racimo. El diseño experimental utilizado fue completamente al azar, en arreglo factorial 2x5, donde el factor 1 fueron los dos híbridos y el factor 2 las dosis de polen, con cuatro repeticiones y nueve inflorescencias/repetición. El peso del racimo fue mayor con la dosis 0.05 g/inflorescencia y menor con la polinización natural. El mayor cuajado de frutos se obtuvo con dosis de 0.1 g/inflorescencia, y el porcentaje de aceite/racimo fue superior con 0.05 g/inflorescencia y menor con 0.01 g/inflorescencia. Los resultados confirman la necesidad de la polinización asistida en los híbridos OxG.

Palabras clave: palma de aceite, fisiología, polinización, cuajado de frutos, potencial de aceite.

Introduction

OxG hybrids are crossbred between American (*Elaeis oleifera*) and African (*Elaeis guineensis*) oil palms and began to be studied in 1970 because some crosses showed resistance to the disease "Bud Rot" (BR), with production over 30 t of bunches/ha/year (Genty and Ujueta, 2013). These hybrids are a valuable alternative for oil production because of their tolerance to pests and diseases, bunch production (25 to 30 t ha^{-1}) and 18% oil extraction (Zambrano, 2004). In addition, their oil has high quality (55% oleic and 11% linoleic acids) and important contents of total vitamin E (tocotrienols 980 mg L^{-1} and tocopherols 100 mg L^{-1}) (Mozzon *et al.*, 2013; Mondragón and Pinilla, 2015) and carotenes (1375-1628 mg L^{-1}) (Rocha *et al.*, 2006; Rivera *et al.*, 2013; Choo and Nesaretnam, 2014).

The hybrid bunches have more parthenocarpic fruits (PF) than normal fruits (NF). The color and oil percentage of red PF are similar to those of NF, but white PF do not have any oil (González *et al.*, 2013). The advantage of hybrid red PF is that they develop and mature like NF, but contain more oil because of their higher proportion of mesocarp, which can reach 98% of the fruit (Bastidas *et al.*, 2007; Preciado *et al.*, 2011a). In addition, the contribution of red PF to the total bunch oil percentage is higher (20-50%) than that of NF (Preciado *et al.*, 2011b).

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Studies on the physiological development of OxG hybrid bunches have shown that the fruits have filling and maturation problems, probably because of asynchronous openings of the flowers or insufficient pollination (Hormaza *et al*., 2012; González *et al*., 2013). In addition, female inflorescences are covered by fibrous spathes that hinder the entry of pollen and pollinators, which translates into a considerable percentage of bunches with aborted fruits (AF) and lower oil potential per bunch (OP) (Zambrano, 2004). For this reason, hybrid plantations employ the practice of assisted pollination, applying *E. guineensis* palm pollen to the inflorescences of hybrid palms to guarantee that female flowers in anthesis receive enough pollen for the development of fruits and reach a reasonable production of bunches (Prada and Romero, 2012; Barcelos *et al*., 2015). The pollen quality depends on the genetic origin, viability, germination capacity and pollen tube growth (Godefroid *et al*., 2010; Shivanna and Tandon, 2014) and is a fundamental requirement in assisted pollination in order to achieve complete fruit set of the bunch.

Haniff and Rosland (2002), in a study on assisted pollination in 12-year-old Tenera palms in Malaysia, evaluated increasing pollen doses (0.0001, 0.001, 0.01, 0.1, 1.0 and 5.0 g/inflorescence) and found that the dose of 0.01 g/inflorescence significantly increased the bunch weight (BW). Since assisted pollination is a very costly agronomic practice for plantations, it is necessary to generate specific management programs for hybrid materials that define the optimal dose of viable pollen in order to guarantee the formation of fruits and reduce costs. The objective of this study was to evaluate the influence of several doses of pollen on fruit development and potential oil production in OxG hybrids.

Materials and methods

Location and plant material

This study was conducted on the OxG hybrids Coarí x La Mé (CxLM) and (Sinú x Coarí) x La Mé (SxCxLM) in an Indupalma experimental field in San Alberto, Cesar, Colombia (10°20' N, 73°11' W, at 125 m a.s.l., maximum temperature 34°C, minimum temperature 22°C, 72% relative humidity, annual precipitation 2,497 mm/year, annual evaporation 1,208 mm and 2,130 h of sunshine/year).

Experimental design

The response of the two hybrids to four 76% germination pollen doses (0.15, 0.10, 0.05, and 0.01 g/inflorescence) and natural pollination as a control was evaluated in a completely randomized design (CRD) with a 2x5 factorial arrangement (factor 1: hybrids and factor 2: pollen doses), four replicates and nine inflorescences/replicate.

Pollination

The pollen used in this experiment was collected in August, 2013 from selected African Tenera palms, in which the male inflorescences were cut in anthesis (stage 607) according to the scale proposed by Hormaza *et al.* (2012) and taken to the Indupalma Seed Laboratory, where the finger-like spines were separated on Kraft paper and dried in a forced convection oven for 12 h at 39°C until 6% humidity; then, they were shaken carefully to release the pollen from the anthers, which was screened with No. 100 and 200 sieves to remove impurities, vacuum packed and stored at -4°C. The pollen quality used in the treatments was determined with the germination test described by Turner and Gilbanks (1974), which counts the percentage of pollen grains that emit a pollen tube; only pollen with 76% germination was used.

The female inflorescences were selected in phenological stage 602 or preanthesis II (Hormaza *et al*., 2012) (Fig. 1A); then, the spathes were removed (Fig. 1B) and covered with insulation bags fitted to the base with an elastic band to avoid contamination from surrounding natural pollen (Fig. 1C). The pollination treatments were carried out when the inflorescences reached the anthesis stage (phenological stage 607), that is, when more than 80% of the flowers had cream-colored receptive stigmas (Hormaza *et al*., 2011) (Fig. 1D). Before the application of the pollen, it was verified that no live insects were inside the bagged female inflorescences to avoid any contamination from foreign pollen; next, the pollen, mixed with inert talc as a transport agent (1:9 ratio), was generously sprinkled through a small hole made with a punch in the plastic window of the isolation bags (Fig. 1E).

The mature bunches were harvested 154 d after pollination, when they naturally detached one to five fruits per bunch (Fig. 1F). The physical analysis of the bunches was done with the methodology described by García and Yáñez (2000) to determine the BW, weight of NF and PF, fruit set coefficient (1) and bunch oil potential (OP) (2) with a subsample of 10 g of dry mesocarp using the Soxhlet method, with hexane as the solvent.

$$\text{Fruit set} = \frac{\text{normal fruits} + \text{parthenocarpic fruits +} + \text{aborted fruits}}{\text{total fruits}} \times 100 \qquad (1)$$

$$\text{Oil potential (\%)} = \text{\% normal fruits oil} + \text{\% parthenocarpic fruits oil} \qquad (2)$$

Statistical analysis

The data were subjected to an analysis of variance (ANOVA) and, for the differences between treatments, the

FIGURE 1. Development stages of the female inflorescences (Hormaza *et al*., 2012). A) pre-anthesis II (phenological stage 602), B) isolated inflorescence, C) enfolded inflorescence, D) anthesis (phenological stage 607), E) pollination, F) mature bunch.

Duncan test ($P<0.05$) was used with the statistical program SAS® (SAS Institute, Cary, NC, USA).

Results and discussion

Bunch weight

Bunch weight (BW) is the sum of the combined weight of spikelets with their individual fruits and the weight of the peduncle or rachis (Mohd, 2000). Figure 2 shows that the higher doses of pollen (0.15, 0.05) produced bunches with a higher weight than the lower doses and natural pollination. Similar trials have also reported a significant increase in BW with doses higher than 0.01 g/inflorescence (Haniff and Rosland, 2002). The natural pollination of the hybrids was very low because of the low emission of male inflorescences that produced a small amount of pollen (5 to 10 g/inflorescence) with low viability (2 to 25%) (Hormaza *et al*., 2012), which was unattractive to the pollinating insect *Elaeidobius kamerunicus* (Tan, 1985).

Fruit set

In the two hybrids, the higher pollen doses provided the largest fruit set, with significant statistical differences when compared to the lowest dose (0.01 g) and the natural pollination control (Fig. 3). The expected result took into account the fact that fruit set depends on the proportion of NF and PF in bunches and, consequently, is directly related to BW, as effectively observed in this study with the higher BW obtained with the high doses. These results agree with those of Haniff and Roslan (2002), who observed a significant increase in BW and fruit set with a pollen dose that was increased from 0.0001 to 0.01 g when using assisted pollination on *E. guineensis* palms.

Fruits/bunch and oil potential

Table 2 shows that there were no significant statistical differences between the hybrids and pollen doses for the NF, PF and AF percentages. However, the tendency for a higher percentage of NF rather than PF was observed with the higher pollen doses. This is a logical consequence of the amount of pollen grains that reach the inflorescence in anthesis, which favors the formation of NF fruits. It has been proven that the assisted pollination of inflorescences in *E. guineensis* palms and OxG hybrids substantially increases the proportion of NF/bunch (Tam, 1981; Rosero and Santacruz, 2014, 2017).

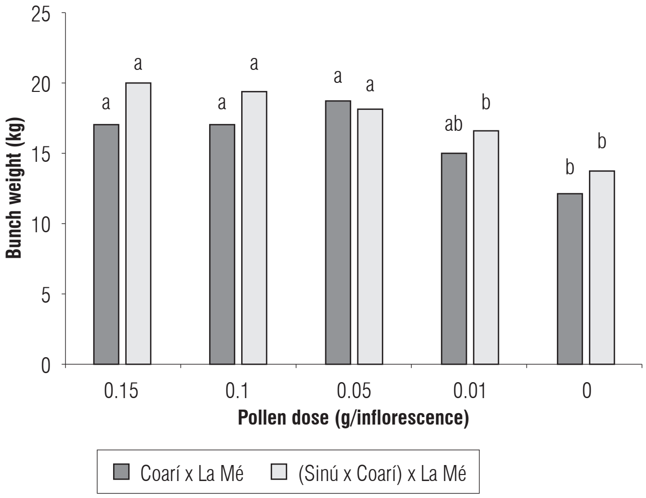

FIGURE 2. Effect of the pollen dose on the bunch weight of two OxG oil palm hybrids. Bars with different letters in each hybrid are statistically different according to the Duncan test ($P<0.05$).

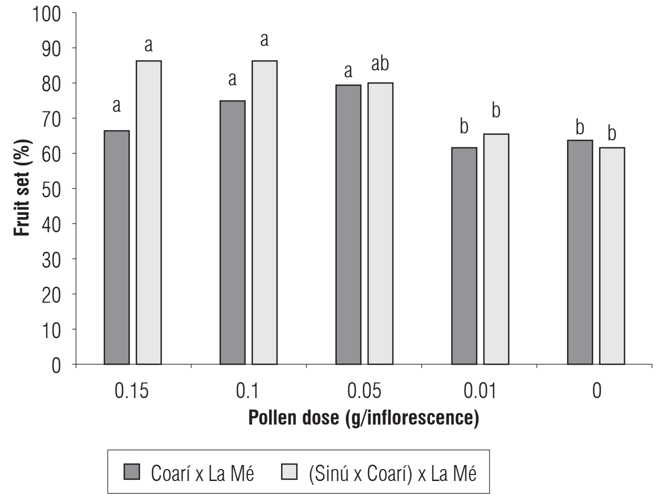

FIGURE 3. Effect of pollen dose on the fruit set of the two oil palm OxG hybrids. Bars with different letters in each hybrid are statistically different according to the Duncan test ($P<0.05$).

TABLE 2. Effect of pollen dose (g/inflorescence) on the normal fruits (NF), parthenocarpic fruits (PF), aborted fruits (AF), and oil potential (OP) in the bunches of the two OxG hybrids.

Hybrid	Dose	Fruits (%)			OP (%)
		NF	PF	AF	
Coarí x La Mé	0.15	44 a	30 ab	10	23.0 ab
	0.1	43 a	35 a	9	25.7 a
	0.05	46 a	29 ab	9	27.0 a
	0.01	31 ab	22 b	11	19.3 b
	Natural pollination	22 b	23 b	8	22.4 ab
(Sinú x Coarí) x La Mé	0.15	49 a	28 a	6	23.4 a
	0.1	45 a	21 b	5	22.1 ab
	0.05	44 a	24 ab	9	20.4 b
	0.01	32 b	21 b	10	21.1 b
	Natural pollination	33 b	16 c	10	18.8 c
CV (%)		30.0	26.5	60.6	24.0
F (Doses)		8.1*	4.2*	0.28 ns	2.2*
F (Hybrids)		1.3 ns	6.1*	0.42 ns	2.64 ns
F (Hybrids x Doses)		0.3 ns	0.37 ns	0.95 ns	0.89 ns

*F test significant ($P<0.05$). ns F test not significant ($P<0.05$)

The higher proportion of PF in hybrid bunches is attributed to the maternal inheritance of the palm *E. oleifera*, characterized by numerous PF that can form up to 90% of the total bunch (Corley and Tinker, 2009), which occurs despite a high percentage of viable pollen (Hardon and Tan, 1969). Zambrano (2004) reported a proportion of between 22 and 49% PF/bunch in OxG hybrids, while Rosero and Santacruz (2017) reported between 44 and 56% PF/bunch. PF increases in bunches have a large impact on the bunch oil potential because the oil content is similar to or even higher than that of NF since the oil in PF pulp can reach up to 98% (Bastidas *et al*., 2011).

The low percentage of AF with all of the pollen doses indicates that the presence of these fruits was not due to the greater or lesser amount of pollen that reached the inflorescences in anthesis, but resulted from adverse factors during the initial development of the bunch fruits. Although the abortion of inflorescences and fruits usually occurs as a consequence of incorrect pollination, some extreme factors, such as water stress, fructification, pruning or shading, can increase it (Corley and Breure, 1992). The AF in the control treatment (natural pollination) were low in number because the experimental field was located between plots of *E. guineensis*, which, most likely, served as a source of pollen for the two hybrids. Hardon and Turner (1967) used natural pollination on *E. guineensis* palm plantations to demonstrate that the majority of pollen remains inside the canopy after emission by male inflorescences, a factor that may favor its transport to the palms.

The OP was higher with the higher doses of pollen (0.15 and 0.10), which showed higher percentages of NF and PF. This coincides with Corley and Tinker (2009), who stated that the variation that exists in OP depends on the proportion of NF and PF in the bunch, which contain oil. In fact, OP indicates the amount of oil that can be extracted per unit of fresh bunch (Moreno *et al*., 2017).

Conclusions

The pollen doses of 0.10 and 0.15 g/inflorescence increased the NF, decreased the AF in the bunches, and favored the production of a higher BW, fruit set, and OP in the two analyzed hybrids.

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A first approach to chalcid wasps (Hymenoptera, Chalcididae) of the entomological museum UNAB with new records for Colombia

Primera aproximación al conocimiento de las avispas calcídidas (Hymenoptera, Chalcididae) del Museo Entomológico UNAB con nuevos reportes para Colombia

Alba Lucía Pérez-Benavides[1] and Francisco Serna[1]*

ABSTRACT

The entomological museum of Universidad Nacional, Faculty of Agricultural Sciences, Bogota, (UNAB) Colombia, conserves several arthropod collections. These collections are considered of great interest to several agricultural and silvicultural production systems in the eastern Andes and the nearby Amazon region (Caqueta and Putumayo provinces) of the country. As a contribution to the knowledge of the diversity of parasitoid wasps associated to agroecosystems (cacao in agroforestry systems, mainly) of these geographical areas, the current research presents a first approach to the study of Chalcididae wasps at this museum. The curatorial status was organized, the represented genera identified, and the geographic occurrences in extension and altitude graphically represented. The Central Taxonomic Collection (CTC) is the main collection at UNAB. It contains 214 specimens of the family Chalcididae that correspond to the genera *Brachymeria, Conura, Dirhinus, Haltichella, Notaspidium, Stypiura, Ecuada,* and *Parastypiura*, with records from 17 provinces. The genera *Ecuada* and *Parastypiura,* as well as 11 species of *Conura,* are recorded for the country for the first time.

Key words: neotropics, taxonomy, geographic occurrence, agroecosystems, parasitoids.

RESUMEN

El museo entomológico de la Universidad Nacional Agronomía Bogotá (UNAB) en Colombia contiene colecciones de artrópodos de importancia en sistemas de producción agrícola y silvícola, provenientes en su mayoría de las regiones de los Andes orientales y la Amazonia cercana del país. Como aporte al conocimiento de la diversidad de avispas parasitoides asociadas a agroecosistemas (cacao en agroforestería, principalmente) de estas áreas geográficas, el presente trabajo presenta una primera aproximación al estudio de las avispas Chalcididae de este museo. Se organizó el estatus curatorial, los géneros representados fueron identificados, y las ocurrencias geográficas en extensión y altitud gráficamente representadas. La Colección Taxonómica Central (CTC) del museo UNAB cuenta con 214 especímenes de la familia, que corresponden a los géneros *Brachymeria, Conura, Dirhinus, Ecuada, Haltichella, Notaspidium, Parastypiura* y *Stypiura*, con registros para 17 departamentos. Los géneros *Ecuada* y *Parastypiura*, así como 11 especies de *Conura* se registran por primera vez para el país.

Palabras clave: neotrópico, taxonomía, ocurrencia geográfica, agroecosistemas, parasitoides.

Introduction

The family Chalcididae (Hymenoptera: Chalcidoidea) contains 1,469 described species including 90 genera (Noyes, 2017). The family is cosmopolitan in distribution, with a higher diversity found in Neotropical lowlands (Delvare and Boucek, 1992; Delvare, 1995), from where 23 genera and 472 species are known (Noyes, 2017). Colombia has five registered subfamilies, including Chalcidinae, Dirhininae, Epitraninae, Haltichellinae, and Smicromorphinae (Boucek, 1988; Delvare, 2004; Noyes, 2017).

Recent studies regarding diversity recognition of Chalcididae from Colombia were carried out under the "Colombia Biodiversa" project (NSF, 2006), cataloging a total of 54 species and nine (9) morphospecies belonging to the following 13 genera: *Aspirrhina, Brachymeria, Conura, Dirhinus, Halsteadium, Haltichella, Hockeria, Hontalia, Melanosmicra, Notaspidium, Psilochalcis, Stypiura,* and *Trigonura*. Likewise, several checklists of Chalcididae from the country have been compiled (Arias and Delvare, 2003; Arias, 2004; Delvare and Arias, 2005). Recently, Noyes (2017) made the Universal Chalcidoidea Database

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available online, reporting 64 species within nine genera from the country, including the genera *Ceyxia, Pilismicra* and *Zavoya*, which were not documented previously.

The entomological museum UNAB (Universidad Nacional Agronomia Bogota) started in 2001 (Vergara *et al.*, 2008; Serna *et al.*, 2017). This museum preserves several arthropod collections of interest to agriculture and forestry production systems mainly from the eastern Andes and nearby Amazon regions from Colombia. It contains approximately 180,000 specimens, including 153,000 pinned or point mounted, 10,430 slide-mounted, 18,400 larvae preserved in ethanol, and 96 Type specimens (Serna *et al.*, 2017). The main objective of this study was to make a first approach to the status of the Chalcididae taxa represented in the UNAB entomological museum, depicting its geographical occurrence.

Materials and methods

Curatorial procedures followed the ones published by Borror *et al.* (1989) and the methodology used was the one established at UNAB by Vergara *et al.* (2008), Martínez and Serna (2015), Malagón-Aldana *et al.* (2017), and Pérez-Benavides *et al.* (2016, 2017). Specimens were point mounted, labeled with information regarding hosts and taxonomic identification, and incorporated into ward boxes with a green cardboard label containing the corresponding taxon name and a catalog number [UNAB #]. Specimen data were digitalized in Excel, including the taxonomy, ecology, and geographical information. Ward boxes containing the specimens were placed into Cornell drawers and compactor shelving cabinets at the Central Taxonomic Collection (CTC).

Morphological characters were studied using a NIKON SMZ-1 stereomicroscope. Photographs were taken with a Canon 5D Mk II camera with EF 100 mm f2.8, incorporating macro lenses, and extensor tubes. For the specimen identification, the taxonomic keys proposed by Burks (1960), Halstead (1990), Delvare (1992, 1992a), Boucek (1956, 1992), and Navarro-Tavares (2013) were used. Identifications were confirmed by reviewing diagnoses and descriptions on the abovementioned publications.

To represent geographical occurrences of the taxa identified, maps were constructed using QGIS (QGIS Development Team, 2015). Complementary data regarding elevations (m: meters above the sea level) were graphically represented. Abbreviations for several data categories recorded on labels are as follows: Fca., small farm; Hda., large farm; No. Catal., Catalog number; Vda., subdivision; Cgto., district; Qda., stream; Ins. Policía: a political division, similar to Cgto (Martínez and Serna, 2015).

Results and discussion

At the UNAB entomological museum, 214 specimens of Chalcididae were found and processed, in total eight genera were found: *Brachymeria, Conura, Dirhinus, Ecuada, Haltichella, Notaspidium, Parastypiura,* and *Stypiura* (Tab. 1).

TABLE 1. Chalcididae taxa represented in the CTC of the entomological museum of UNAB and number of specimens found per taxon.

TAXA	Number of specimens at the CTC in UNAB
CHALCIDINAE	**207**
Conura	178
Brachymeria	25
Stypiura	3
Parastypiura	1
HALTICHELLINAE	**8**
Dirhinus	2
Ecuada	1
Haltichella	3
Notaspidium	2

Geographic occurrence

Specimens of Chalcididae in the CTC at UNAB were collected mostly in the Southwestern and Central regions of Colombia. The results confirm that this family is found in 52 municipalities of the country, in the provinces of Amazonas, Antioquia, Arauca, Caldas, Caqueta, Casanare, Cesar, Cundinamarca, La Guajira, Meta, Nariño, Putumayo, Quindio, Risaralda, Santander, Tolima, and Valle del Cauca. The genus *Conura* (Figs. 1A and B) corresponds to the most widely distributed taxon and contains the largest number of specimens in the Chalcid collection.

Furthermore, genera records from each province are as follows: Cundinamarca: *Conura, Brachymeria,* and *Dirhinus* (Fig. 1A-D), Caqueta: *Conura, Brachymeria, Notaspidium, Haltichella,* and *Stypiura* (Fig. 1A-D), Caldas: *Ecuada* (Fig. 1D), and Antioquia: *Parastypiura* (Fig. 1D).

Altitude distribution

Chalcididae specimens were found in a gradient from 8 to 2,150 m a.s.l. (Fig. 2), with an average of 378 m a.s.l., agreeing with the records published by Delvare (1995), who attributed a higher distribution of this family in Neotropical lowland areas. *Conura* was collected from 8 to 1,949 m

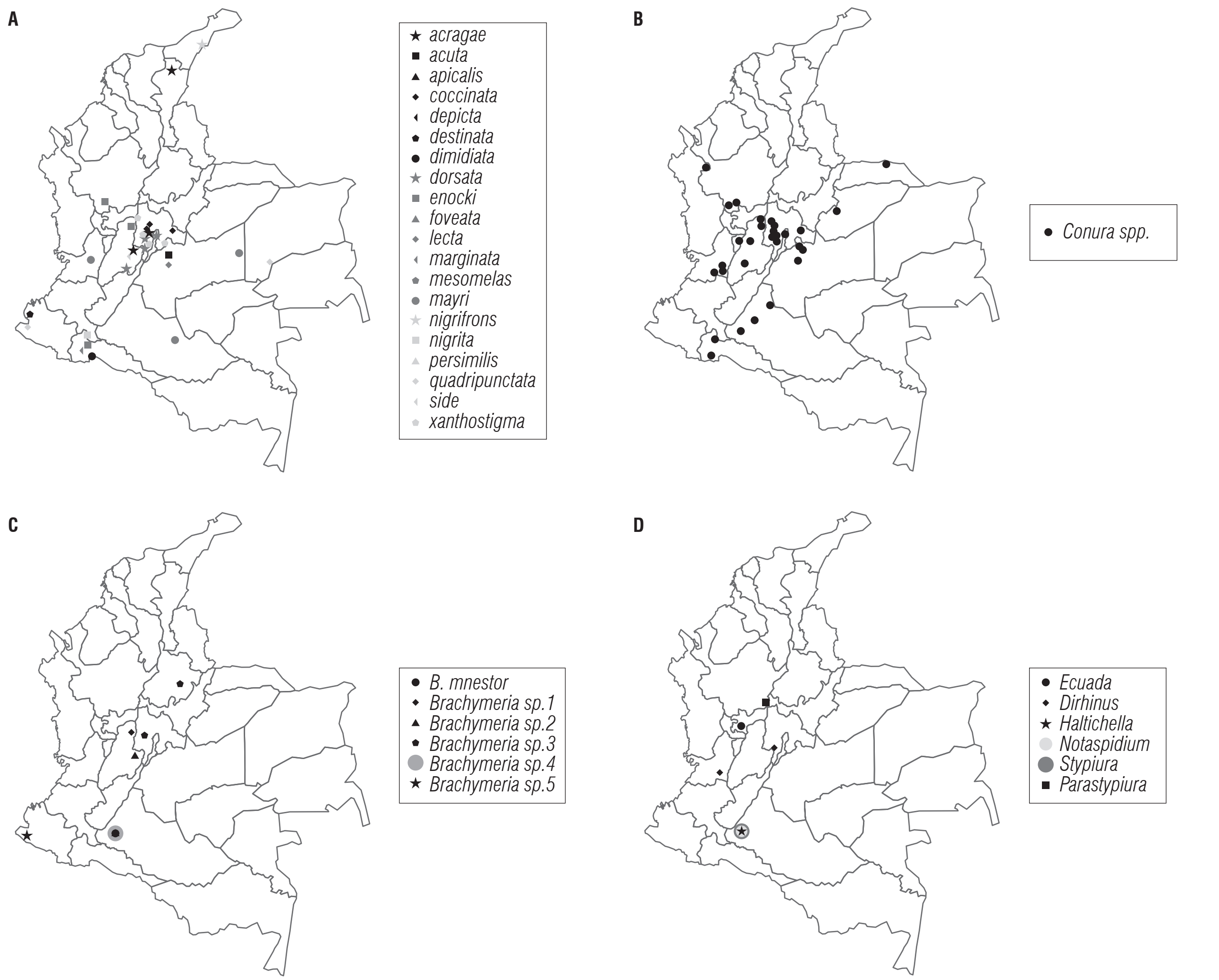

FIGURE 1. Geographic occurrence of Chalcididae in Colombia represented in the CTC of UNAB. A) species of *Conura*, B) morphospecies of *Conura*, C) species and morphospecies of *Brachymeria*, D) genera *Ecuada, Dirhinus, Haltichella, Notaspidium, Stypiura,* and *Parastypiura*.

a.s.l., with an average of 467 m a.s.l., and *Brachymeria* was found from 13 to 2,046 m a.s.l., with an average of 302 m a.s.l. These genera were more often collected in agroforestry areas. Further assessments showed that the genera *Dirhinus, Ecuada, Haltichella, Notaspidium, Parastypiura* and *Stypiura* were restricted to more specific elevations (Fig. 2), and specimens are not common in collections. *Ecuada* was found up to 2,150 m a.s.l., contrasting with the much narrower elevation range reported by Boucek (1992) from 400 up to 850 m a.s.l.

Worldwide, *Conura* and *Brachymeria* are the richest genera in terms of species described within the family, accounting for 303 and 313, respectively (Noyes, 2017). However, identification of species into these genera is difficult. In the literature, taxonomic identification keys are scarce and incomplete, and numerous current descriptions are in

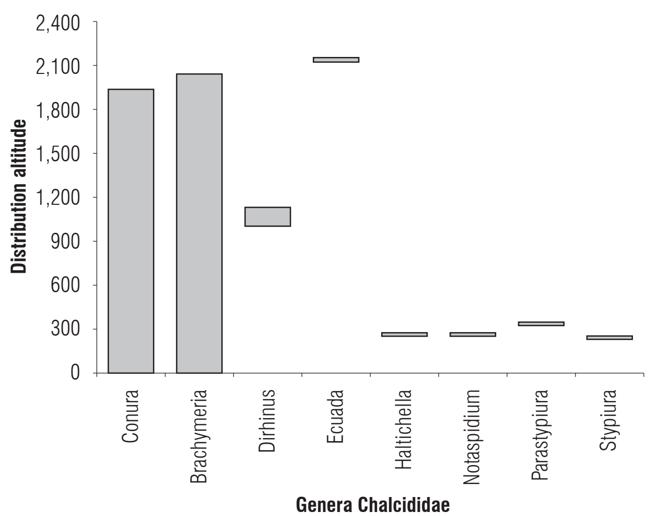

FIGURE 2. Elevation ranges (distribution altitude, m a.s.l.) of genera Chalcididae from Colombia represented in the CTC of UNAB.

need of taxonomic revisions. For the genus *Brachymeria*, Burks (1960) and Delvare (1992) offer identification keys for species commonly found in the Neotropical region.

Nonetheless, Delvare (1992) provided a key for the species groups of *Conura*, being currently the most comprehensive tool for species identification within this genus. This author recorded 75 species in the *Conura* genus for Colombia (the Noyes checklist 2017, and "the Insect Survey of a Megadiverse Country: Colombia" - National Science Foundation, NSF 2006), including *acragae, adela, aequalis, amoena, annulipes, apaiis, apicalis, ashmilis, attacta, belti, biannulata, bipunctata, blanda, camescens, carinata, compactilis, contributa convexa, correcta, cressoni, dares, debilis, decisa, delicata, depicta, desmieri, destinata, discolor, distincta, dorsimaculata, eubule, expleta, femorata, ferruginea, flava, flavicans, foveata, fulvovariegata, fusiformis, hirtifemora, immaculata, incongrua, lecta, lenkoi, lobata, maculata, maculipennis, magdelenensis, marcosensis, masus, media, miniata, morleyi, nigrifrons, nigrita, odontotae, oiketicusi, paya, philippia, pintoi, pseudofulvovariegata, pygmaea, quadrilineata, quadripunctata, rasplusi, rufoscutellaris, side, surumuae, toluca, transidiata, transitiva, tygen, unilineata, vau*, and *xanthostigma*.

Despite this wide survey, the results obtained from the UNAB survey increased the abovementioned checklist in 11 species, including *acuminata, acuta, albomaculata, coccinata, dimidiata, dorsata, enocki, mayri, mesomelas, marginata*, and *persimilis*, for a total list of 86 *Conura* species reported for Colombia.

Based on published checklists, Chalcids from Colombia comprise a total of 15 genera and 102 species (Fernández 1995; NSF 2006; Noyes 2017). However, in the UNAB entomological museum some specimens represent new records for Colombia. Among these, the genera *Ecuada* and *Parastypiura,* as well as records of 11 species of the genus *Conura,* updated the checklist of Chalcididae for Colombia to 17 genera and 115 species. Furthermore, regarding Chalcididae published-collections containing curated-voucher specimens (point mounted, labeled, identified, and cataloged), the central taxonomical collection of the UNAB entomological museum constitutes the largest Chalcididae collection in Colombia. Authors such as Fernández (1995), NSF (2006), and Noyes (2017) highlight that most Chalcididae specimens representing previously published inventories from Colombia have been deposited into collections outside the country. Hence, representative Colombian Chalcididae collections in the country are scarce, and, therefore, it is important to compile, update, and disseminate the information included in these collections.

The status of the Chalcididae subfamily deposited in the central taxonomic collection of the entomological museum of UNAB, including the number of specimens, geographical data in Colombia and new records for the country are listed below.

CHALCIDINAE

Conura Spinola, 1837

Conura acragae Delvare, 1993

Material examined: 4 specimens. **Caldas**, Belalcazar, Hda. Beltran, 900 m, 4°57' N, 75°47' W, 25-Sep-2010, J.M. Perilla [**UNAB** 3526]. **Tolima**, Espinal, 323 m, 4°9' N, 74°56' W, 9-Jun-1979, F. Riveros [**UNAB** 3526]. **Cundinamarca**, La Mesa, 1,298 m, 4°38'0.5" N, 74°27'57" W, 12-Sep-2003, C. Matiz [**UNAB** 3526]. **Cesar**, Valledupar, Fca. San Miguel, 169 m, 10°27' N, 73°15' W, 6-Apr-2012, G. Villamizar [**UNAB** 3526].

Conura acuminata (Ashmead, 1904)

Material examined: 1 specimen. **Meta**, Villavicencio, Universidad de los Llanos, 467 m, 4°04' N, 73°34' W, 20-Sep-2012, R. Tejedor [**UNAB** 1034].

Conura acuta (Fabricius, 1804)

Material examined: 2 specimens. **Tolima**, Carmen de Apicala, 328 m, 4°8' N, 74°43' W, 27-Jul-1994, A. Torres [**UNAB** 3528]. **Meta**, Guamal, 525 m, 3°52' N, 73°46' W, 29-Mar-1994, J.G. Ortiz [**UNAB** 3528].

Conura albomaculata (Ashmead, 1904)

Material examined: 1 specimen. **Caqueta**, San Vicente del Caguan, Vda. Palestro, Fca. El Limonar, 270 m, 2°7'11.40" N, 74°45'0.82" W, 30-Mar-2016, L. Pérez [**UNAB** 1865].

Conura apicalis (Ashmead, 1904)

Material examined: 2 specimens. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 20-Mar-2015, L. Pérez; J. Martínez [**UNAB** 1872]. **Cundinamarca**, Sasaima, 1,203 m, 4°57' N, 74°26' W, 28-Sep-1975, L.H. Ramirez [**UNAB** 1872].

Conura coccinata (Cresson, 1872)

Material examined: 4 specimens. **Cundinamarca**, La Vega, Laguna El Tabacal, 1,337 m, 5°1' N, 74°19' W, 12-Aug-2012, C. Sosa [**UNAB** 1035]. **Cundinamarca**, La Vega, Vda. El Vino, 1,230 m, 5°1' N, 74°20' W, 30-Sep-2011, J. Cante [**UNAB** 1035]. **Cundinamarca**, La Mesa, Vda. San Javier, 1,142 m, 4°40’ N, 74°27’ W, 1-Jun-1997, A. Alessandri [**UNAB** 1035]. **Cundinamarca**, Ubala, Vda. El Puerto, 1,949 m, 4°44’ N, 73°32’ W, 20-Jan-1998, M. García [**UNAB** 1035].

Conura decisa (Walker, 1862)

Material examined: 9 specimens. **Arauca**, Arauca, Caño Limon, 125 m, 7°5’ N, 70°45’ W, 12-Jun-1990, H. Urrego [**UNAB** 1886]. **Tolima**, Natagaima, 323 m, 3°37’ N, 75°5’ W, 1-May-1996, Moncaleano [**UNAB** 1886]. **Cundinamarca**, Ricaurte, 284 m, 4°16’ N, 74° 46’ W, 30-Sep-1972, M. Ospina [**UNAB** 1886]. **Cundinamarca**, Villeta, 829 m, 5°0’51” N, 74°28’33” W, 2-Jun-2014, A. Becerra [**UNAB** 1886]. **Meta**, Puerto Lopez, Fca. Hato grande, 184 m, 4°5› N, 72°57’ W, 19-Oct-2012, J. Carrasco [**UNAB** 1886]. **Putumayo**, Villa Garzon, Vda. San Fidel, Fca. La Cuca, 362 m, 0°50’15” N, 76°38’5.9” W, 25-Mar-2015, D. Gómez [**UNAB** 1886]. **Putumayo**, Mocoa, Vda. Rumiyaco, Fca. Heraldo Vallejo, 700 m, 1°07’48.6” N, 76°39’55.8” W, 4-Mar-2016, L. Vargas [**UNAB** 1886]. **Putumayo**, Mocoa, Vda. Caliyaco, Tropical Amazonian Botanical Garden, 584 m, 1°7’6” N, 76°37’58” W, 18-Sep-2015, D. Jurado [**UNAB** 1886]. **Putumayo**, Villa Garzon, Vda. San Rafael, Fca. El Escondite, 317 m, 0°47’41.6” N, 76°35’82” W, 29-feb-2016, C. Mora [**UNAB** 1886].

Conura depicta (Walker, 1864)

Material examined: 1 specimen. **Nariño**, Tumaco, Vda. Cajapi, Km 30 via Tumaco-Pasto, UNAL, 10 m, 1°36’36.2” N, 78°43’12.8” W, 22-Sep-2015, L. Pérez [**UNAB** 1871].

Conura destinata (Walker, 1864)

Material examined: 1 specimen. **Nariño**, Francisco Pizarro, Salahonda, 8 m, 2°02’14” N, 78°39’30.56” W, 4-Mar-2016, E. Ortegón [**UNAB** 1887].

Conura dimidiata (Fabricius, 1804)

Material examined: 3 specimens. **Valle del Cauca**, Yotoco, Vda. Muñecos, Cgto. El Dorado, Fca. la Union, 1,522 m, 3°51’ N, 76°26’ W, 27-Nov-2013, S. Calderón [**UNAB** 1032]. **Putumayo**, Villa Garzon, Vda. San Rafael, Fca. El Escondite, 317 m, 0°47’42” N, 73°35’8” W, 19-Sep-2015, K. Rodriguez [**UNAB** 1032]. **Putumayo**, Puerto Asis, Vda. Brisas de Hong Kong, 270 m, 0°28’55.6” N, 76°30’17.7” W, 25-Mar-2015, S. Córdoba [**UNAB** 1032].

Conura dorsata (Cresson, 1872)

Material examined: 5 specimens. **Tolima**, Natagaima, Vda. Yaco, 349 m, 3°29’ N, 75°10’ W, 2-Jun-2014, J. Rojas [**UNAB** 1889]. **Tolima**, Melgar, Villa Sofia neighborhood, 349 m, 4°12’ N, 74°37’ W, 1-Mar-2014, O. Cristancho [**UNAB** 1889]. **Cundinamarca**, La Vega, 1,217 m, 4°38’9.87” N, 74°5’19.56” W, 11-May-2014, A. Gamba [**UNAB** 1889]. **Cundinamarca**, Anapoima, 716 m, 4°33’ N, 74°32’ W, 6-Jan-2014, E. Fernandez [**UNAB** 1889]. **Risaralda**, Santa Rosa de Cabal, Vda. El Guayabal, Fca. Yoyaricuma, 1,750 m, 4°87’ N, 75°62’ W, 10-May-2012, E. Quintero [**UNAB** 1889].

Conura enocki (Ashmead, 1904)

Material examined: 3 specimens. **Antioquia**, Tarso, Vda. Condor, Fca. Peña Bonita, 1,266 m, 5°52’1” N, 75°49’1” W, 23-Sep-2012, J. Perilla [**UNAB** 1866]. **Tolima**, Piedras, Hda. El Chaco, 426 m, 4°57’ N, 74°53’ W, 5-Oct-2012, L. Buitrago [**UNAB** 1866]**. Putumayo**, Villa Garzon, Vda. San Fidel, Fca. La Cuca, 352 m, 0°50’15” N, 76°38’5.9” W, 27-Mar-2015, S. García [**UNAB** 1866].

Conura foveata (Kirby, 1883)

Material examined: 1 specimen. **Tolima**, Melgar, 323 m, 4°12’ N, 74°38’ W, 9-Jul-1978, A. Sánchez [**UNAB** 1888].

Conura lecta (Cresson, 1872)

Material examined: 1 specimen. **Meta**, Granada, 345 m, 3°33’34” N, 73°42’21” W, 1-Nov-2015, D. Guevara [**UNAB** 3562].

Conura marginata (Ashmead, 1904)

Material examined: 1 specimen. **Putumayo**, Orito, Vda. El Yarumo, km 35, Fca. El Limonar, 325 m, 0°39’36.7” N, 76°47’24.1” W, 26-Mar-2015, S. García [**UNAB** 3527].

Conura mayri (Ashmead, 1904)

Material examined: 1 specimen. **Meta**, Puerto Gaitan, Fca. La Fazenda, 149 m, 3°50’ N, 71°15’ W, 28-May-2009, J. García; I. Quiroga [**UNAB** 1869].

Conura mesomelas (Walker, 1862)

Material examined: 1 specimen. **Putumayo**, Mocoa, Vda. Caliyaco, Jardin Botanico Tropical Amazonico, 584 m, 1°7’6” N, 76°37’58” W, 18-Sep-2015, A. Pantoja [**UNAB** 3465].

Conura nigrifrons (Cameron, 1884)

Material examined: 1 specimen. **La Guajira**, Maicao, 52 m, 11°22’ N, 72°14’ W, 4-Dec-1995, J. Vargas [**UNAB** 3558].

Conura persimilis (Ashmead, 1904)

Material examined: 1 specimen. **Tolima**, Natagaima, Vda. Yaco, 349 m, 3°29’ N, 75°10’ W, 2-Jun-2014, J. Rojas [**UNAB** 1867].

Conura nigrita (Howard, 1894)

Material examined: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11’28.1” N, 76°38’48.3” W, 20-Mar-2015, L. Pérez [**UNAB** 3429].

Conura quadripunctata (Fabricius, 1804)

Material examined: 3 specimens. **Nariño**, Tumaco, Vda. Cajapi, Km 30 vía Tumaco-Pasto, UNAL, 10 m, 1°36›36.2" N, 78°43'12.8" W, 22-Sep-2015, L. Pérez [**UNAB** 1868]. **Cundinamarca**, Fusagasuga, 1,728 m, 4°20' N, 74°21' W, 20-Apr-2000, J. Otálora [**UNAB** 1868]. **Amazonas**, Leticia, Parque Nacional Natural Amacayacu (Amacayacu Natural National Park), 100 m, 3°29' N, 70°12' W, 12-Jun-1995, F. Fernández [**UNAB** 1868].

Conura side (Walker, 1843)

Material examined: 1 specimen. **Tolima**, Saldaña, Vda. Santa Marta, Qda. Papayal, 410 m, 3°56' N, 75°1' W, 4-Apr-2002, J. Martínez [**UNAB** 1870].

Conura xanthostigma (Dalman, 1820)

Material examined: 9 specimens. **Cundinamarca**, La Mesa, 1,198 m, 4°9' N, 72°60' W, 11-Nov-2012, L. Zubieta [**UNAB** 1037]. **Cundinamarca**, Puente Quetame, 1,361 m, 4°18' N, 73°51' W, 24-Nov-2012, L. Guerrero [**UNAB** 1037]. **Cundinamarca**, Sasaima, Vda. Santa Ana, 1,376 m, 4°55' N, 74°26' W, 17-May-2014, T. Cárdenas [**UNAB** 1037]. **Cundinamarca**, Anolaima, 1,657 m, 4°46' N, 74°28' W, 2-Nov-2012, D. Ramírez [**UNAB** 1037]. **Cundinamarca**, Tibacuy, Vda. La Vuelta, 1,600 m, 4°20' N, 74°27' W, 1-May-2010, R. Forero [**UNAB** 1037]. **Cundinamarca**, Cachipay, Laguna Verde, 1,600 m, 4°743' N, 74°26' W, 21-Feb-1998, V. Bernal; K. Turriago [**UNAB** 1037]. **Cundinamarca**, Quipile, Vda. El Retiro, 1,400 m, 4°39' N, 74°35' W, 14-Jun-2015, S. García [**UNAB** 1037]. **Tolima**, Honda, 214 m, 5°12'11" N, 74°44'17" W, 27-Mar-2010, D. Salinas [**UNAB** 1037]. **Valle del Cauca**, Yotoco, Vda. Muñecos, Cgto. El Dorado, Fca. La unión, 1,522 m, 3°50'49.84" N, 76°26'24.5" W, 27-Nov-2013, L. Pérez [**UNAB** 1037].

Conura spp.

***Conura* sp.1**: 14 specimens. **Cundinamarca**, El Colegio, Margen occidental del rio Cauca (west margin of the Cauca river), 990 m, 4°35' N, 74°27' W, 1-Sep-2012, F. Ariza [**UNAB** 1033]. **Cundinamarca**, Silvania, 1,470 m, 4°24' N, 74°23' W, 15-Mar-1997, C. R. Bojacá [**UNAB** 1033]. **Cundinamarca**, La Mesa, 1,200 m, 4°37'49" N, 74°27'45" W, 30-Apr-2011, D. López [**UNAB** 1033]. **Antioquia**, Puente Iglesias, 569 m, 5°49' N, 75°42' W, 14-Apr-2012, J. Ordoñez [**UNAB** 1033]. **Antioquia**, Betania, 1,550 m, 5°44' N, 75°58' W, 10-Jan-1994, A. Avella [**UNAB** 1033]. **Arauca**, Arauca, Caño Limon, 125 m, 7°5' N, 70°45' W, 12-Jun-1990, Y. Urrego [**UNAB** 1033]. **Caqueta**, Florencia, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 12-Sep-2014, L. Pérez [**UNAB** 1033]. **Meta**, Villavicencio, Universidad de los Llanos (Unillanos), 467 m, 4°9'12" N, 73°38'06" W, 11-May-2011, A. Romero [**UNAB** 1033]. **Putumayo**, Mocoa, Vda. Rumiyaco, Fca. Heraldo Vallejo, 700 m, 1°07'48.6" N, 76°39'55.8" W, 4-Mar-2016, M. Vanegas [**UNAB** 1033]. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 700 m, 1°11'28.1" N, 76°38'48" W, 20-Mar-2015, L. Pérez [**UNAB** 1033]. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 700 m, 1°11'28.1" N, 76°38'48" W, 28-Feb-2016, L. Riveros [**UNAB** 1033]. **Putumayo**, Orito, Vda. El Yarumo, km 35, Fca. El Limonar, 325 m, 0°39'36.7" N, 76°47'24.1" W, 26-Mar-2015, J. Martínez [**UNAB** 1033]. **Quindío**, Armenia, 1,900 m, 4°32' N, 75°40' W, 18-Oct-2001, A. Sarmiento [**UNAB** 1033]. ***Conura* sp.2**: 4 specimens. **Meta**, Villavicencio, 467 m, 4°15' N, 73°64' W, 17-Oct-2012, L. Zubieta [**UNAB** 1036]. **Cundinamarca**, San Juan de Rioseco, La Mesita, 1,303 m, 4°37' N, 74°21' W, 24-May-2012, C. Pinilla [**UNAB** 1036]. **Cundinamarca**, Guaduas, 992 m, 5°4' N, 74°34' W, 14-Oct-2012, A. Gámez [**UNAB** 1036]. **Caqueta**, Florencia, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 12-Sep-2014, L. Perez [**UNAB** 1036]. ***Conura* sp.3**: 1 specimen. **Meta**, Villavicencio, Ins. Policia La Cuncia, 1,420 m, 4°04' N, 73°32' W, 19-Sep-2012, N. Tejedor [**UNAB** 1038]. ***Conura* sp.4**: 1 specimen. **Antioquia**, Puente Iglesias, west margin of the Cauca river, 569 m, 5°49' N, 75°42' W, 13-Mar-2012, C. Pinilla [**UNAB** 1039]. ***Conura* sp.5**: Material examined: 1 specimen. **Cundinamarca**, Anapoima, 710 m, 4°33' N, 74°32' W, 4-Mar-2012, C. Pinilla [**UNAB** 1040]. ***Conura* sp.6**: 1 specimen. **Santander**, Puente Nacional Nogales, 331 m, 4°57' N, 74°54' W, 12-Oct-2012, S. Dussán [**UNAB** 1349]. ***Conura* sp.7**: 2 specimens. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1350]. ***Conura* sp.8**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1351]. ***Conura* sp.9**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 257 m, 1°30'37' N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1352]. ***Conura* sp.10**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1353]. ***Conura* sp.11**: 1 specimen. **Caqueta**, El Doncello, Vda. Anayacito, Granja Copoazu, 337 m, 1°40'2.33" N, 75°16'57" W, 1-Sep-2014, F. Serna [**UNAB** 1354]. ***Conura* sp.12**: 1 specimen. **Meta**, Villavicencio, 467 m, 4°15' N, 73°64' W, 17-Oct-2012, L. Zubieta [**UNAB** 3530]. ***Conura* sp.13**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37' N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 3531]. ***Conura* sp.14**: Material examined: 5 specimens. **Caqueta**, Florencia, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 12-Sep-2014, L. Pérez [**UNAB** 3532]. ***Conura* sp.15**: 1 specimen. **Caqueta**, El Doncello, Vda. Anayacito, Granja Copoazu, 337 m, 1°40'2.33" N, 75°16'57" W, 22-Sep-2014, M. Bermúdez [**UNAB** 3533]. ***Conura* sp.16**: 9 specimens. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Mar-2016, L. Pérez [**UNAB** 3534]. Endoparasitoid on Pieridae chrysalid on plants of *Duranta* sp. (Verbenaceae). ***Conura* sp.17**: 1 specimen. **Putumayo**, Mocoa, Vda. Rumiyaco, Fca. Heraldo Vallejo, 700 m, 1°07'48.6" N, 76°39'55.8" W, 4-Mar-2016, D. Cárdenas [**UNAB** 3535]. ***Conura* sp.18**: 2 specimens. **Putumayo**, Orito, Vda. El Yarumo km 35, Fca. El Limonar, 325 m, 0°39'36.7" N, 76°47'24.1" W, 26-Mar-2015, J. Martínez [**UNAB** 3536]. ***Conura* sp.19**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 12-Sep-2014, M. Bermúdez [**UNAB** 3537]. ***Conura* sp.20**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 20-Mar-2015, L. Pérez [**UNAB** 3538]. ***Conura* sp.21**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 22-Mar-2015, L. Pérez [**UNAB** 3539]. ***Conura* sp.22**: 1 specimen. **Putumayo**, Mocoa, Vda. Caliyaco, Tropical Amazonian Botanical Garden, 584 m., 1°7'6" N, 76°37'58.7" W, 18-Sep-2015, A. Pantoja [**UNAB** 3540]. ***Conura* sp.23**: 1 specimen. **Caqueta**, San Vicente del Caguan, Vda. Palestro, Fca. El Limonar, 270 m, 2°7'11.40" N, 74°45'0.82" W, 20-Mar-2016, L. Pérez [**UNAB** 3541]. ***Conura* sp.24**: 1 specimen. **Putumayo**, Orito, Vda. El Yarumo, km 35, Fca. El Limonar, 325 m, 0°39'36.7" N, 76°47'24.1" W, 26-Mar-2015, N. Pérez [**UNAB** 3542]. ***Conura* sp.25**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 22-Mar-2015, L. Pérez [**UNAB** 3543]. ***Conura* sp.26**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 22-Mar-2015, L. Pérez [**UNAB** 3544]. ***Conura* sp.27**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 13-Oct-2014, M. Bermúdez [**UNAB** 3545]. ***Conura* sp.28**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 21-Mar-2015, J. Martínez [**UNAB** 3546]. ***Conura* sp.29**: 1 specimen. **Amazonas**, Leticia, Km 22, via Tarapacá, 82 m, 4°07'12" N, 69°56'26" W, 18-Aug-1997, D. Campos [**UNAB** 3547]. ***Conura* sp.30**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 620 m, 1°11'28.1" N, 76°38'48.3" W, 18-Sep-2015, J. Jiménez [**UNAB** 3548]. ***Conura* sp.31**: 1 specimen. **Putumayo**, Mocoa, Vda. Pueblo Viejo, Fca. Villa Loca, 352 m, 1°11'28.1" N, 76°38'48.3" W, 20-Mar-2015, L. Pérez [**UNAB** 3549]. ***Conura* sp.32**: 1 specimen. **Casanare**, Yopal, Utopia campus, Universidad de La Salle, 249 m, 5°19'33.4" N, 72°17'47.4" W, 7-Aug-2015, H. Córdoba [**UNAB** 3550]. ***Conura* sp.33**: 4 specimens. **Cundinamarca**, Villeta, 801 m, 5°00'11.9" N, 74°28'41.6" W, 16-May-2010, M. Pineda [**UNAB** 3551]. **Cundinamarca**, Villeta, 850 m, 5°00' N, 74°28' W, 5-May-1991, C. Navia [**UNAB** 3551]. **Cundinamarca**, Mesitas, Vda. San Jose, 983 m, 4°35'14" N, 74°26'58" W, 4-Dec-2004, S. Cubillos [**UNAB** 3551]. **Tolima**, Ibague, El Rodeo, 1,250 m, 4°28' N, 75°18' W, 8-May-2004, O. Guataquira [**UNAB** 3551]. ***Conura* sp.34**: 3 specimens. **Valle del Cauca**, Santiago de Cali, 995 m, 3°26' N, 76°31' W, 1-Apr-1971, G. Cayón [**UNAB** 3553]. **Valle del Cauca**, El Cerrito, Semillas del Valle S.A, 987 m, 3°41'1" N, 76°19'30" W, 14-Oct-2003, E. Ortiz [**UNAB** 3553]. **Tolima**, Chaparral, Barrio Beltran (Beltran neighborhood), Granja Sixto Iriarte (Sixto Iriarte Farm), 854 m, 3°43'39" N, 75°29'16" W, 27-Aug-2003, A. Diaz [**UNAB** 3553]. ***Conura* sp.35**: 1 specimen. **Meta**, San Martin, Fca. El Caduceo, 405 m, 3°41'1" N, 73°41' W, 5-Jun-2012, J. Rodriguez [**UNAB** 3554]. ***Conura* sp.36**: 1 specimen. **Tolima**, Mariquita, Cataratas del rio Medina (Medina river waterfalls), 495 m, 5°11' N, 74°53' W, 16-Nov-1999, Lic. Biologia (Biology bachelor) [**UNAB** 3555]. ***Conura* sp.37**: 2 specimens. **Tolima**, Ibague, Vda. La Esperanza, 1,000 m, 4°28' N, 75°18' W, 18-Nov-2003, O. Guataquira [**UNAB** 3556]. **Antioquia**, Puente Iglesias, Margen occidental del rio Cauca (west margin of the Cauca river), 569 m, 5°49'54" N, 75°42'37" W, 13-Apr-2012, O. Ortiz [**UNAB** 3556]. ***Conura* sp.38**: *Conura* sp.38, 1 specimen. **Cundinamarca**, Ubala, Vda. El Puerto, 1,949 m, 4°44' N, 73°32' W, 20-Jan-1998, M. García [**UNAB** 3557]. ***Conura* sp.39**: 1 specimen. **Amazonas**, Leticia, Km 22 via Tarapaca, 82 m, 4°07'12" N, 69°56'26" W, 18-Aug-1997, D. Campos [**UNAB** 3559]. ***Conura* sp.40**: 2 specimens. **Cundinamarca**, Mesitas, Vda. San Jose, 983 m, 4°35'14" N, 74°26'58" W, 4-Dec-2004, S. Cubillos [**UNAB** 3560]. **Cundinamarca**, Anolaima, 1,607 m, 4°46'00" N, 74°28'00" W, 23-Mar-2011, A. Silva [**UNAB** 3560]. ***Conura* sp.41**: 1 specimen. **Antioquia**, Dabeiba, Bomba "Terpel" ("Terpel" fuel station), 395 m, 7°00' N, 76°39' W, 29-Mar-2014, J. Velásquez [**UNAB** 3561]. ***Conura* sp.42**: 5 specimens. **Valle del Cauca**, Palmira, Instituto Colombiano Agropecuario (ICA) (Colombian Agriculture Institute), 1,001 m, 3°32' N, 76°17' W, 12-Jun-1905, L. Nuñez [**UNAB** 3563]. **Valle del Cauca**, Santiago de Cali, zoologico de Cali (Cali zoo), 1,035 m, 3°26' N, 76°33' W, 27-Oct-2014, W. Hernández [**UNAB** 3563]. **Cundinamarca**, La Vega, 1,217 m, 4°38' N, 74°5'19.56" W, 11-May-2014, A. Gamba [**UNAB** 3563]. **Cundinamarca**, Anapoima, 710 m, 4°33' N, 74°32' W, 10-Nov-2012, F. Ariza [**UNAB** 3563].

Cundinamarca, Sasaima, 1,376 m, 4°55' N, 74°26' W, 23-Oct-1990, Rodríguez [**UNAB** 3563].

Brachymeria mnestor (Walker, 1841)

Material examined: 2 specimens. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Mar-2016, L. Pérez [**UNAB** 3525].

Comments from the authors: several Pieridae chrysalids in *Duranta* sp. (Verbenaceae) plants were collected and then placed into breeding chambers. From these, a single parasitoid of *B. mnestor* emerged from each of the two chrysalids.

Brachymeria spp.

***Brachymeria* sp.1**: 6 specimens. **Santander**, Puente Nacional, Nogales, 331 m, 4°57' N, 74°54' W, 12-Oct-2012, S. Dussán [**UNAB** 1041]. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 257 m, 1°29'59" N, 75°39'47" W, 25-Nov-2014, C. Motta [**UNAB** 1041]. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1041]. ***Brachymeria* sp.2**: 7 specimens. **Tolima**, Espinal, Corpoica Nataima, 420 m, 4°9' N, 74°53' W, 23-Apr-2012, M. Cárdenas [**UNAB** 1345]. **Caqueta**, Florencia, Macagual Amazonian Research Center, 274 m., 1°29'58.70" N, 75°39'46.44" W, 12-Sep-2014, L. Perez [**UNAB** 1345]. **Caqueta**, El Doncello, Vda. Anayacito, 337 m, 1°40'2.33" N, 75°16'57" W, Sep-2014, F. Serna [**UNAB** 1345]. ***Brachymeria* sp.3**: 8 specimens. **Cundinamarca**, Anolaima, Vda. San Rafael, 2,046 m, 4°49' N, 74°26' W, 13-Sep-2012, J. Rivera [**UNAB** 1346]. **Santander**, San Gil, 1,117 m, 6°33' N, 73°8' W, 3-Oct-2012, D. Ramírez [**UNAB** 1346]. ***Brachymeria* sp.4**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75° 39'46.44" W, 12-Sep-2014, L. Pérez [**UNAB** 1348]. ***Brachymeria* sp.5**: 1 specimen. **Nariño**, Tumaco, Vda. Cajapi, Km 30, via Tumaco-Pasto, 13 m, 1°36'36.2" N, 78°43'12.8" W, 22-Sep-2015, L. Pérez [**UNAB** 1817].

Stypiura sp.

Material examined: 3 specimens. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 233 m, 1°30'37" N, 75°40'21" W, 25-Oct-2014, M. Bermúdez [**UNAB** 1356]. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m., N 1°29'58.7", W 75°39›46.44", 2-Sep-2016, L. Pérez [**UNAB** 1356].

Parastypiura sp. (Figs. 3A and B)

Material examined: 1 specimen. **Antioquia**, Puerto Triunfo, Reserva Natural cañon del Rio Claro (Natural Reserve of the Claro River Canyon), 350 m, 5°52' N, 74°38' W, 1-Jun-1985, R. Vélez [**UNAB** 1823].

Comment by the authors: this is the first record of the genus for Colombia.

FIGURE 3. Specimen of the genus *Parastypiura* from the CTC of the UNAB entomological museum: A) lateral view, B) dorsal view.

FIGURE 4. Specimen of the *Ecuada producta* from the CTC of the UNAB entomological museum: A) lateral view, B) dorsal view, C) frontal view.

HALTICHELLINAE

Dirhinus sp.

Material examined: 1 specimen. **Cundinamarca**, Arbelaez, Vda. Hato Viejo, Fca. Parcela, 1,140 m, 4°50'1.5" N -74°27'38.2" W, 22-Mar-2016, D. Cubillos [**UNAB** 3552].

Dirhinus (*Dirhinus*) sp.

Material examined: 1 specimen. **Valle del Cauca**, Palmira, Corpoica, Banco de Germoplasma (Germplasm Bank), 1,003 m, 3°31'47" N, 76°18'13" W, 15-Oct-2003, E. Ortiz [**UNAB** 1824].

Ecuada producta Boucek, 1992 (Figs. 4A-C)

Material examined: 1 specimen. **Caldas**, Manizales, Jardin Botanico (Botanical Garden of Manizales), 2,150 m, 5°6'15" N, 75°33'10" W, 29-Mar-2010, D. Salinas [**UNAB** 3529].

Comment by the authors: this is the first record of the genus for Colombia.

Haltichella ornaticornis Cameron, 1884

Material examined: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 2-Sep-2016, L. Pérez [**UNAB** 2530].

Haltichella spp.

***Haltichella* sp.1**: Material examined: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 2-Sep-2016, L. Pérez [**UNAB** 2531]. ***Haltichella* sp.2**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 2-Sep-2016, L. Pérez [**UNAB** 2532]. ***Haltichella* sp.3**: 1 specimen. **Caqueta**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 13-Sep-2014, L. Pérez [**UNAB** 1190].

Notaspidium sp.

Material examined: 1 specimen. **Caquetá**, Florencia, Vda. La Viciosa, Macagual Amazonian Research Center, 274 m, 1°29'58.70" N, 75°39'46.44" W, 13-Sep-2014, L. Pérez [**UNAB** 1189].

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Physicochemical characteristics of cacao (*Theobroma cacao* L.) soils in Colombia: Are they adequate to improve productivity?

Características fisicoquímicas de los suelos con cacao (*Theobroma cacao* L.) en Colombia: ¿Están adecuados para mejorar la productividad?

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ABSTRACT

In Colombia, cacao farms are located mainly in areas with edaphoclimatic limitations. The predominant soil fertility characteristics from small cacao farms in 13 provinces were evaluated. A total of 635 soil samples (0-20 cm) were taken between 2011 and 2013 from the main cacao producer regions in Colombia. The number of samples was assigned based on the regional cacao production. The resulting data were analyzed with a linear regression model using pH as the dependent variable and soil chemical and physical parameters as the independent variables. Soil texture ranged between 14 and 40% clay, and 31 and 65% sand. Bulk density ranged between 1.07 and 1.28 Mg m^{-3} with a calculated porosity between 48.8 and 55.0%. There was no evidence of soil compaction. The national pH ranged between 3.9 and 7.9 and was negatively correlated with Al^{+3} content (R^2 = 0.68) and Al saturation (R^2 = 0.80) and redicts Al saturation in the range of available Al up to pH >5.2-5.5. In this range, available P (as P_2O_5) was lower than 12 mg kg^{-1} and was accepted as a minimum value for a sustainable production. With 4.0 $cmol_c$ kg^{-1} of Ca and 60% saturation of the effective cation exchange capacity (ECEC). A minimum ECEC is proposed at 6.0 $cmol_c$ kg^{-1}. A reference table for soils in Colombia is proposed as a guide for the establishment and management of productive and sustainable plantations.

Key words: yield, fertilization, mineral nutrition, zoning.

RESUMEN

En Colombia, el cultivo de cacao está establecido en su mayoría en áreas con limitaciones edafoclimáticas. Se evaluaron las condiciones prevalentes de fertilidad de los suelos en 13 departamentos tomando 635 muestras durante 2011 al 2013, de las condiciones químico-físicas del suelo (0-20 cm) en las principales zonas productoras de Colombia asignando el número de muestras según la producción regional. Los datos fueron analizados mediante un modelo de regresión lineal utilizando como variable dependiente el pH y las independientes las variables químicas y físicas del suelo. La textura varió entre 14 y 40% de arcilla y 31 y 65% de arena. La densidad aparente varió entre 1.07 y 1.28 Mg m^{-3} con porosidad total calculada entre 48,8 y 55% sin problemas de compactación. El pH al nivel nacional varió entre 3.9 y 7.9, y estuvo negativamente correlacionado con el contenido de Al $^{+3}$ (R^2 = 0.68), y la saturación de Al (R^2 = 0.80) y predice linealmente la saturación de Al en el rango de Al disponible en pH >5.2-5.5. En este rango de pH el P disponible (as $P_2O_{5)}$ no superó 12 mg kg^{-1}, que es el nivel sugerido como el mínimo requerido para producciones sostenibles. Con 4.0 $cmol_c$ kg^{-1} de Ca y 60% de saturación de la CICE se propone una CICE mínima de 6.0 $cmol_c$ kg^{-1}. Se propone una tabla de referencia como guía para el establecimiento y el manejo de plantaciones productivas y sostenibles.

Palabras clave: producción, fertilización, nutrición mineral, zonificación.

Introduction

In Colombia, cacao plantations have a significant socioeconomic importance because they are cultivated in rural areas with high levels of poverty and the prevalence of social conflicts. Traditionally, cacao beans are not only commercialized but are also consumed as part of the rural and urban diets, even more than coffee (García-Cáceres *et al.,* 2014). Cacao production depends on family labor and its cultivation and processing is generally an additional activity from a diversified farm system and might not be the main economic activity (Espinal *et al.,* 2005; Petterson, 2016). However, it is estimated that more than 52,500 families depend directly on cacao for their livelihood (Baquero, 2018). The Colombian government expects this cropping system to generate a significant contribution to the illicit crop substitution process that is currently being carried out.

Although nearly 660,000 ha have been identified for planting cacao without any agricultural restriction, cacao must compete with other crops for prime areas and is generally not planted (García-Lozano, 2004; García-Cáceres, 2014).

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Thus, this crop is usually planted in areas with edaphoclimatic limitations that may restrict its productive potential (García-Lozano, 2004; Méndez *et al.,* 2006). Limitations include low water or nutrient availability, soil acidity, and mechanization difficulties, among others. These constraints partially explain the low national yields which do not exceed 500 kg ha^{-1}/yr, despite research and development efforts made in the country by research and developmental institutions (Agrosavia and universities) and private commercial entities (Fedecacao, Casa Luker, Nutresa). Available planting materials do not express their potential productivity because small and medium-scale producers do not perform timely fertilization (Álvarez-Carrillo *et al*., 2015), pest or disease management (Jaimes *et al*., 2011). The price of sustained fertilizers increases over the years, which discourages their use (Álvarez-Carrillo *et al*., 2015).

Despite the lack of management, cacao in Colombia is mostly classified as fine or flavored (Fedecacao, 2019) and recognized in annual showcase events, such as the *Salon du Chocolat* in Paris, France (IICO, 2016). This privileged condition, which only applies to 5% of the world cocoa production, is one more reason to promote and increase bean production. Application of fertilizers and soil amendments to improve production is essential to make the cacao cultivation more profitable and sustainable. The main objective of this study was to propose benchmark soil fertility conditions for Colombia as a guide for the establishment and maintenance of productive and sustainable plantations.

Materials and methods

A national cacao production survey was carried out between 2011 and 2013 in the largest cacao producing areas in Colombia. Based on the information collected with this survey, a physicochemical soil sampling scheme (0 to 20 cm depth) was carried out in productive commercial crops of at least more than three years of age. The highest cacao producing municipalities are located in 14 provinces: Antioquia, Arauca, Bolivar, Boyaca, Cesar, Cordoba, Guajira, Huila, Magdalena, Meta, Nariño, Norte de Santander, Santander, and Tolima.

Statistical analysis

Sample size and statistical distribution were established according to the planted area per province. Sampling points selected per municipality, and sample size per province were considered in the equation for finite populations (planted area per province) (Eq. 1).

$$n_1 = \frac{N^* z_\alpha^2 \rho^* q}{d^2 \times (N-1) + Z_\alpha^2 \times p \times q_1} \quad (1)$$

where:

N = Total population

Zα = 1.96 (with 95% confidence)

P = desired proportion (in this case 5% = 0.05)

q = 1-p (in this case 1-0.05 = 0.95)

d = precision (5%)

The sample sites were established using data from municipal agricultural evaluations (EVAS) carried out by MADR in 2011 (MADR, 2016). The numbers of samples assigned per provinces/municipalities are shown in Table 1. Six municipalities of Santander that represented the biggest cacao producing province were analyzed based on their specific weight in the national production. In total 635 cacao farms were sampled.

TABLE 1. Distribution of the number of samples per municipality and province.

Province	Municipality	Samples (*n*)
Santander	San Vicente	68
	Rio Negro	58
	Landazuri	49
	El Carmen	44
	Playon	14
	Cimitarra	11
Arauca	Arauquita, Saravena, Tame	59
Antioquia	Apartado, Arboletes, Caracoli, Chigorodo, Maceo, San juan de Uraba, Turbo	44
Nariño	Tumaco	40
Norte de Santander	Sardinata, Tibu	39
Tolima	Ataco, Chaparral, Cunday, Falan, Icononzo, Villarica	34
Magdalena	Santa Marta	26
Meta	Cubarral, El Dorado, San Juan de Arama, Vista Hermosa, Fuente de Oro, Granada	23
Huila	Garzon, Gigante, Palermo, Rivera, Tesalia	22
Cesar	Manaure	15
Boyaca	Borbur, Maripí, Muzo, Pauna	12
Cordoba	Los Cordobas, Tierralta, Valencia	11
Bolivar	Santa Rosa	10
Guajira	Dibuya	9
TOTAL		635

Sampling and laboratory analysis

Soil samples were taken from productive cacao plantations with a minimum of 3 years of establishment and yields higher than 600 kg ha^{-1} of dry cacao. Plots were selected with areas not bigger than 5 ha (average for cacao producers). Sampling was carried out in the soil area with the highest root density: the cacao plant crown (De Almeida and Valle, 2007) in plots of 50 m x 50 m, with at least four subsamples.

Physicochemical variables were analyzed at Agrosavia facilities, according to the following methods: pH (potentiometric method, soil-water ratio 1:2.5), exchangeable acidity (KCl 1N), interchangeable Al, texture by touch, organic matter (modified Walkley Black), P (Bray II), S (monocalcium phosphate), interchangeable bases (Ca, Mg, K, in 1M of ammonium acetate at pH 7) and ECEC (effective cationic exchange capacity by addition of cations). Moreover, bulk density was determined by undisturbed sampling with a known volume ring, and the particle density was obtained using the pycnometer method.

Statistical analysis

The soil data were analyzed using a linear regression model design applying a normality test. The pH range of the Colombian soils was used against the physicochemical soil properties as the dependent variable, adjusting the model using the R© software.

Results and discussion

The scope of this study corresponds to 635 soil samples (0-20 cm) taken between 2011 and 2013, each one from a productive cacao field, generally no bigger than 5 ha. Sampling was carried out in 13 of the 23 provinces in Colombia, representing the largest cacao producing areas in the country. Since six municipalities in Santander (El Carmen, Rio Negro, El Playon, Landazuri, Cimitarra and San Vicente) accounted for 32% of the cacao production in the country, each one was treated as if it was a province in the following graphs and tables (Tab. 1).

Cacao plantations ranged from 0 to 1,400 m a.s.l. (Fig. 1) and are considered representative of areas where cacao can be planted in Colombia. Depending on topography, there are provinces such as Antioquia and Norte de Santander where cacao is cultivated throughout the range of altitude, while in others (Nariño, Meta and Arauca) it is cultivated at a low altitude and relatively flat topographies. However, most of the cacao farms are located between 350 and 1,000 m a.s.l. (Fig. 1).

Most of these plantations are established in udic to mildly ustic soil moisture regimes. Additionally, they loosely correspond to forest biomes ranging from humid to dry tropical forest and/or well drained river terraces of mixed mineralogy, as they are located all along a north-south transect of the Andes Mountains of Colombia with very different parent materials. Plantation locations vary widely, but due to farmers' knowledge, they seemed to follow natural patterns of higher soil fertility rather than land availability. For example, in the province of Meta, despite having a very large quantity of available land, cacao farmers preferred a thin strip of higher fertility and better drained alluvial terraces (RV soil series) surrounding the Ariari river rather than the dominant acid soils in a piedmont landscape (PV soil series) and well drained plateaus dominated by acid soils (LV soil series) (Fig. 2).

Soil texture (per province) ranged between 14 and 40% clay, and between 31 and 65% sand. Silt was the least variable

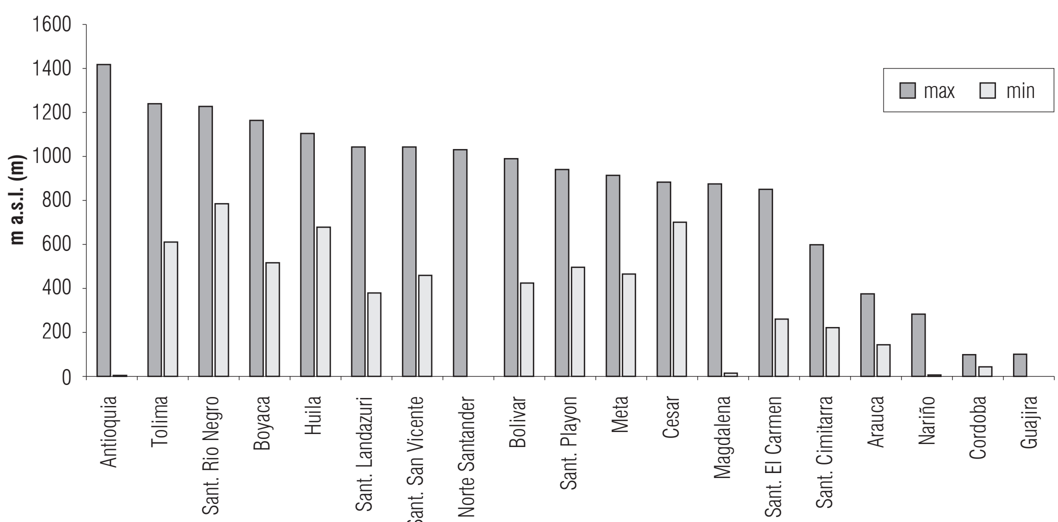

FIGURE 1. Altitudinal range (m a.s.l.) of the main cacao growing areas per province in Colombia.

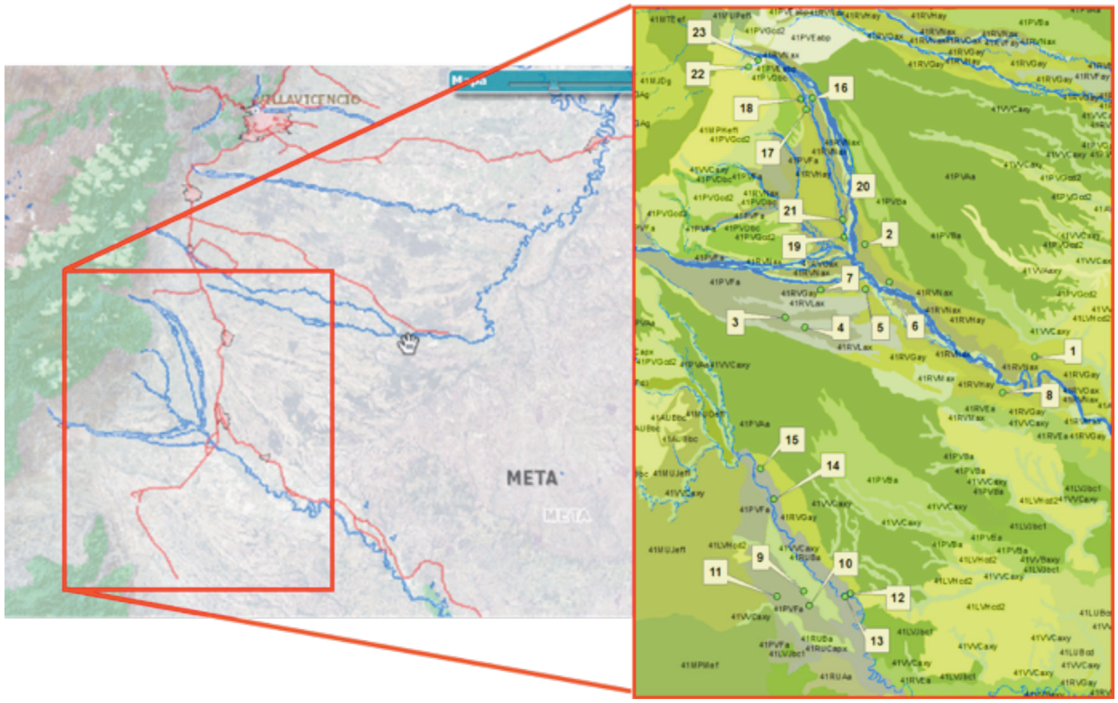

FIGURE 2. Spatial distribution of cacao farms sampled in the municipality of Granada, Meta, Colombia.

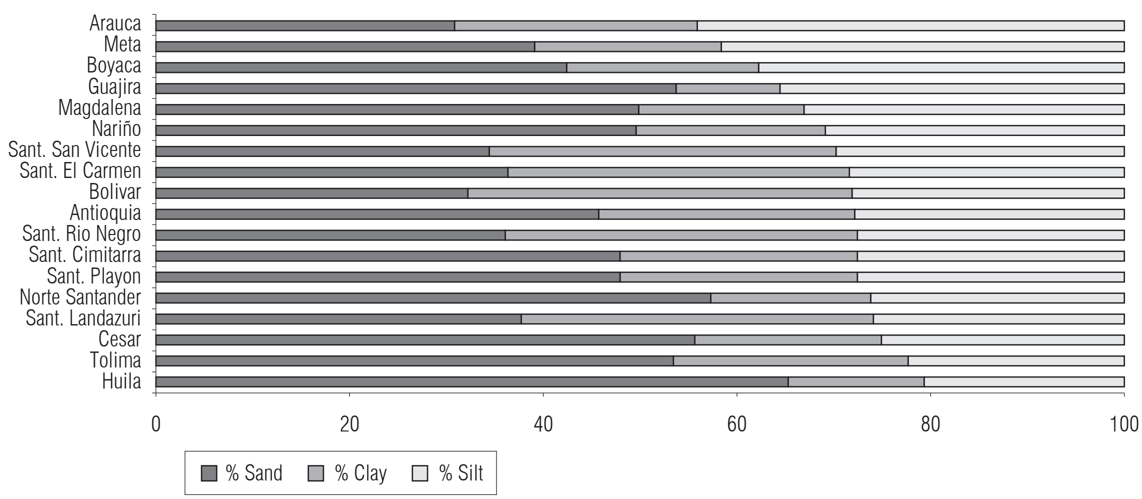

FIGURE 3. Average soil surface textures (0-20 cm, arranged by silt content decrease) of productive farms in the provinces/municipalities with the highest cacao production rates in Colombia from 2011 to 2013.

textural component ranging between 20 and 44%. Predominant soil textures were loamy, clay-loam, sand-loam and loamy-sandy-clay (Fig. 3). With these textural ranges, real density averaged between 2.36 and 2.59 mg m^{-3} and bulk density between 1.07 and 1.28 mg m^{-3}. Total porosity varied in a narrow range between 48.8 and 55.0%, with no evidence of compaction (data not shown). Mean SOM contents varied between 1.5 and 4.9% (Tab. 2), although the national value ranged between 0.3 and 13%. Only 28 samples had SOM values lower than 1%, and five samples showed values higher than 10%, mainly in Landazuri.

The average physicochemical soil properties by province/municipality arranged by increasing mean pH are shown in Table 2. Because in Colombia small-scale producers were found to rarely use soil amendments or fertilization practices, a fact corroborated by the survey, and typical of small cacao farmers worldwide (Snoeck *et al.*, 2016; Van Vliet *et al.*, 2017), the values presented are representative of soils without mayor amendments or fertilizer practices.

Reference soil nutrient content proposed for cacao in Colombia (García-Lozano *et al.,* 2004) and other producing countries (Wessel, 1971; Paramananthan, 2000; Snoeck *et al.*, 2016) are shown in Table 3. These reference values are meant as a guide to intensify cacao productivity.

The correlation matrix coefficients for soil chemical properties and calculated % saturations of Colombian soils are shown in Table 4. Of the evaluated parameters, pH, Al^{+3} and Ca^{+2} and their saturations showed the highest correlations. However, base and Al saturation (as percent of the ECEC) consistently showed the best correlation with coefficients greater than 75%.

Statistical analysis indicated that the data were adjusted to normality assumptions for the generation of parametric linear models. As an example, the adjustment to the pH vs. Al saturation model shows that it is comprised of two parts: a linear adjustment with pH values<5.5, and a portion without adjustment for pH>5.5. For the linear portion, the atypical points suggested by the model were eliminated from the regression (8 points of 357 for the linear portion) (Fig. 4) and the correlation coefficient of $R^2 = 0.7626$ is improved to 0.7964. In this case, the linear model changes the intercept (pH) from 5.2070 to 5.2162, and the Al saturation coefficient (X) changed from -0.01175 to -0.0120.

The pH is one of the most indicative variables of the general condition of the soil (Fageria and Baligar, 2008). On average, the pH of soils under cacao per province varied from 4.9 to 6.1, but nationally ranged between 3.9 and 7.9 (Tab. 2). Some provinces such as Huila, Guajira and Magdalena showed a very narrow pH range, while Santander, Tolima, Antioquia and Meta showed pH ranges higher than 2.4-3.5 in relatively small regions, which are similar in magnitude to the national values (Fig. 5). Only Bolivar, Meta, Santander/El Carmen, Santander/Rio Negro and Boyaca had mean province values lower than 5.2, which indicate fields prone to high aluminum content. As an indicator, a pH>5.0 seems reasonable for Colombia allowing for some aluminum tolerance for the currently available materials.

The available phosphorus content (Bray II) in the country was between 0.1 and 334.9 mg kg^{-1}. Province range varied between 2.7 and 67.2 mg kg^{-1} (Tab. 2). No correlation was found between available P content and pH ($R^2 = 0.068$) or

TABLE 2. Average physicochemical properties of cacao productive areas in Colombia from 2011 to 2013.

Province	PD	BD	Porosity	Sand	Clay	SOM	pH	P	Al	Ca	Mg	K	ECEC
	mg m^{-3}		%					mg kg^{-1}		cmol$_c$ kg^{-1}			
Bolivar	2.36	1.11	53.00	32.34	39.80	2.80	4.93	2.71	2.45	2.58	0.90	0.31	6.33
Meta	2.59	1.19	53.98	39.21	19.28	2.32	5.00	33.08	0.72	2.82	0.54	0.13	4.72
Sant./ El Carmen	2.50	1.17	53.11	36.64	35.06	3,03	5.00	26.97	2.58	5.23	0.87	0.15	8.92
Sant./ Rio Negro	2.49	1.23	50.39	36.16	36.54	2,6.	5.15	9.94	1.71	3.20	1.16	0.17	6.37
Boyaca	2.48	1.12	55.01	42.50	19.87	3.02	5.17	17.21	1.25	4.93	1.27	0.19	7.73
Norte de Santander	2.45	1.25	48.85	57.39	16.77	2.22	5.23	11.30	0.82	4.96	1.23	0.16	7.22
Arauca	2.48	1.19	51.91	30.92	25.05	1.48	5.25	15.94	0.37	3.81	1.70	0.14	6.08
Sant./ Playon	2.49	1.26	49.27	47.80	24.91	2.23	5.27	15.79	0.81	2.93	1.43	0.13	5.40
Sant./ Cimitarra	2.56	1.16	49.27	34.18	35.09	2.76	5.36	8.39	5.97	5.97	1.25	0.14	8.68
Sant./ Landazuri	2.37	1.16	51.26	37.88	36.48	4.84	5.33	67.16	1.56	6.53	1.41	0.19	9.80
Tolima	2,37	1.17	50.71	53.67	24.20	3.50	5.36	12.95	1.08	6.17	2.15	0.32	9.77
Antioquia	2.53	1.13	55.40	45.69	26.73	1.65	5.47	7.67	0.41	8.12	3.52	0.24	12.39
Sant./ San Vicente	2.46	1.18	51.94	34.64	35.92	2.62	5.55	28.85	2.01	9.41	0.99	0.21	12.71
Nariño	2.35	1.07	54.51	49.91	19.28	3.20	5.95	5.23	0.11	5.29	2.03	0.16	7.73
Cordoba						2.53	5.98	8.64	0.27	11.08	4.79	0.35	16.71
Magdalena				50.07	17.20	3.52	6.10	46.64	0.02	7.74	3.07	0.13	11.26
Cesar				55.65	19.55	3.57	6.16	58.68	0.00	7.53	1.51	0.20	9.33
Huila	2.49	1.28	48.75	65.42	14.24	2.50	6.16	13.60	0.01	5.92	2.35	0.20	8.59
Guajira				53.64	11.07	3.05	6.32	45.01	0.00	7.18	2.01	0.14	9.54

BD: bulk density, PD: particle density, SOM: soil organic matter, ECEC: effective cation exchange capacity.

another chemical parameter. Moreover, of the total number of samples taken, only 47 samples had levels below 2 mg kg^{-1} (Fig. 6) and 25 samples had levels higher than 108 mg kg^{-1} (not shown in Fig. 6). For Colombia, a minimum of 12 mg kg^{-1} of available P is proposed, which corresponds approximately to the maximum level of P found in soils with some Al content (pH<5.5) (Tab. 5).

Our proposal fits the limits suggested for other countries (Tab. 3). From the samples analyzed, 64.4% showed some level of deficiency. On average, seven municipalities/provinces showed values below the suggested levels. In these cases, P fertilizer application is required to obtain a competitive production.

TABLE 3. Reference chemical parameter levels for cacao soils in Colombia and other countries.

Parameter	Unit	García-Lozano *et al.* (2004)	Paramananthan (2000)	Snoeck *et al.* (2016)	Wessel (1971)
pH		5.0	5.0	5.1	5.5
Organic C	%		1.5	1.7	1.75
SOM		>3.0	2.57	2.93	3.02
P	$\mu g\ g^{-1}$	13*	15	6/12**	12/24***
Al	$cmol_c\ kg^{-1}$	<40		>1.5	0.0
Ca		>8.0		4.0	8.0
Mg		1	0.25	0.9	2.0
K		0.3	0.25	0.2	0.2
ECEC			12	12	

SOM: soil organic matter

*13 $\mu g\ g^{-1}$ calculated based on an apparent density of 1.3 mg m^{-3} with 58 kg ha^{-1} of P_2O_5.

**Melick/Olsen extraction.

***sandy-clay soils.

TABLE 4. Correlation matrix of the chemical parameters evaluated for cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013.

	pH	Al^{+3}	Ca^{+2}	Mg^{+2}	K^{+}	Ca^{+2}+ Mg^{+2}	ECEC
pH	1.000	-0.627	0.743	0.379	0.185	0.738	0.566
Al^{+3}		1.000	-0.536	-0.449	-0.154	-0.580	-0.078
Ca^{+2}			1.000	0.422	0.316	0.971	0.912
Mg^{+2}				1.000	0.351	0.627	0.567
K^{+}					1.000	0.364	0.391
Ca^{+2}+ Mg^{+2}						1.000	0.933
ECEC							1.000

	pH	Al sat.	Ca sat.	Mg sat.	K sat.	Sat. Ca, Mg	Sat. bases
pH	1.000	-0.747	0.768	0.229	-0.178	0.754	0.747
Al sat.		1.000	-0.902	-0.533	0.066	-0.996	-0.999
Ca sat.			1.000	0.128	-0.188	0.912	0.905
Mg sat.				1.000	0.033	0.524	0.531
K sat.					1.000	-0.148	-0.075
Sat. Ca + Mg						1.000	0.997
Sat. bases							1.000

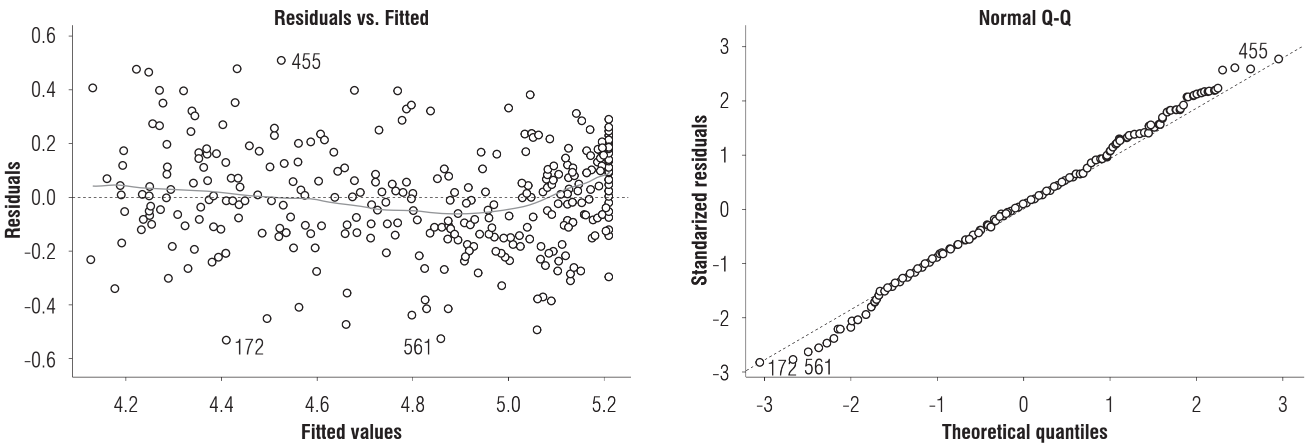

FIGURE 4. Normality assumptions of the data ($n = 635$) for the regression model of the relationship between pH and Al saturation in Colombian cacao soils.

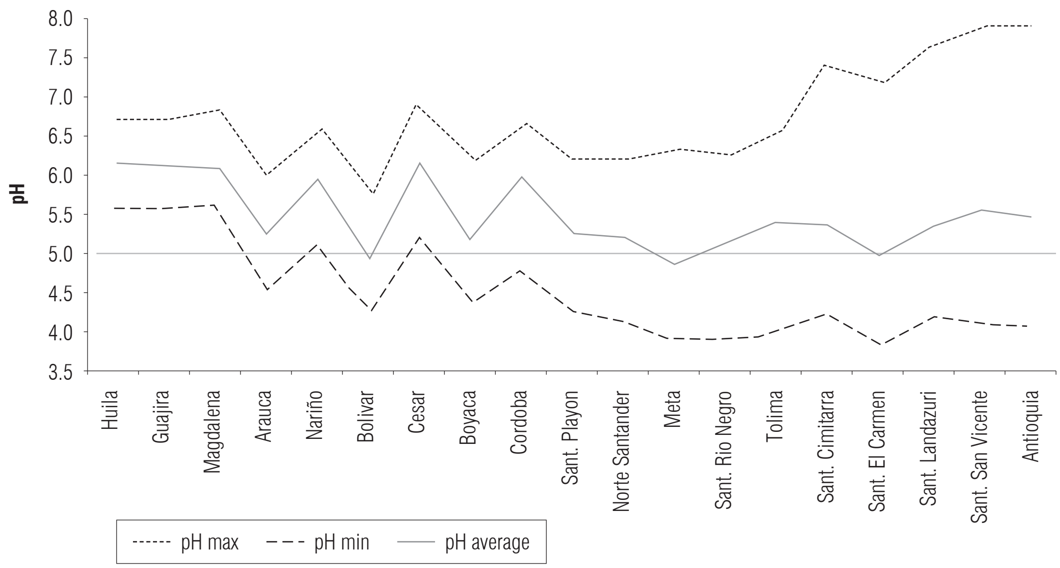

FIGURE 5. pH range of the soils under representative cacao productive areas cultivated by small-scale farmers in Colombia (ordered from low to high range) from 2011 to 2013.

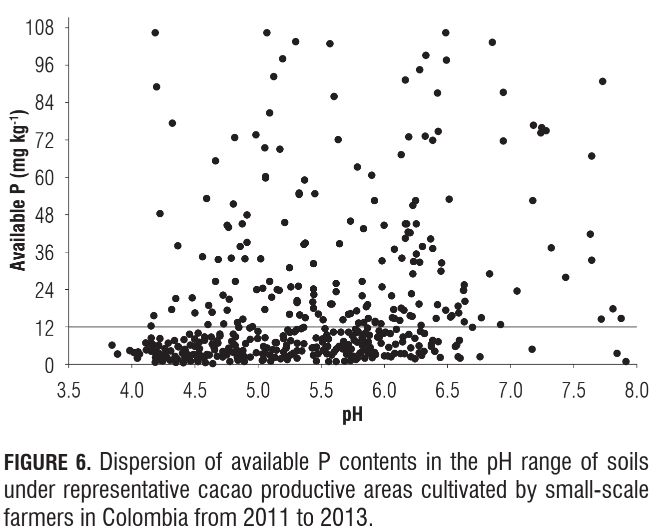

FIGURE 6. Dispersion of available P contents in the pH range of soils under representative cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013.

Al^{+3} saturation was highly correlated with soil pH rather than with the available Al content (R^2 = -0.747 vs. -0.627 respectively, Tab. 4) (Fageria and Baligar, 2008). From the national model of pH vs % Al saturation (R^2 = -0.828, Tab. 5), 76% of the model linearly predicts Al content in the range of pH>5.2-5.5, (Al% = 346.04 - 65.022*pH). For higher pH (pH<5.5), no correlation was found (R^2 = 0.0152) (Fig. 7).

We propose that for soils with <20% of Al^{+3} saturation, equivalent to >1.5 $cmol_c$ kg^{-1} of available Al^{+3}, and with genetic materials with low Al^{+3} susceptibility, damage should not be expected at the root level, and additional amendments may not be required for adequate production at least in the initial years (Ribeiro *et al.*, 2013). However, amending the Al^{+3} component will improve the efficiency of applied

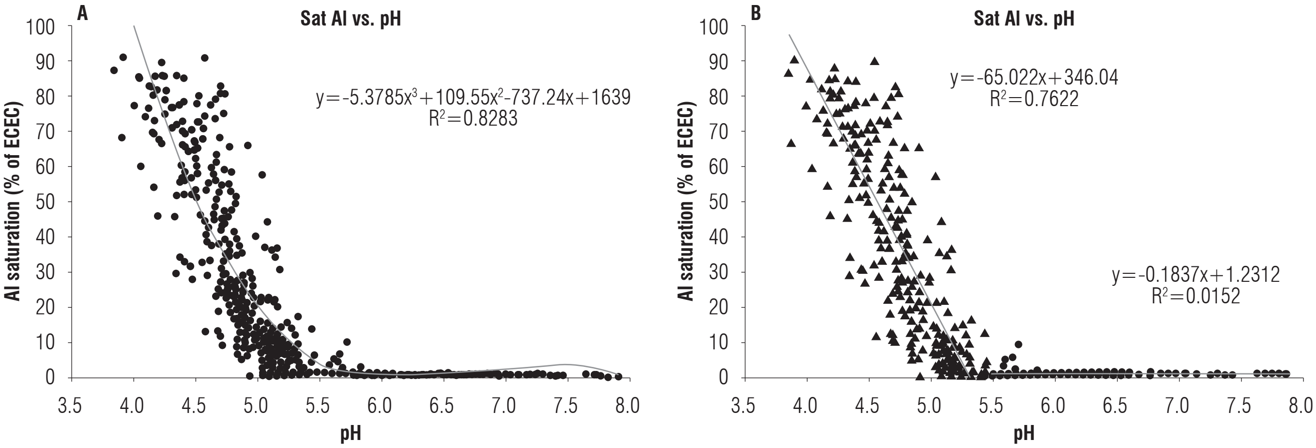

FIGURE 7. Relationship between pH and Al saturation under representative cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013: a) model of full pH range; b) two phase model as per %Al content at pH=5.5.

fertilization (Puentes-Páramo *et al*., 2014; Rosas-Patiño *et al*., 2017) in Typic Udorthents soils of the Colombian Amazon, dolomite lime was applied at levels of 7 mg ha^{-1} which reduced the exchangeable Al^{+3} levels improving nutrient availability and productivity. A minimum pH value of 5.0 is proposed as a predictor of a chemical condition of the soil for cacao cultivation in Colombia (Tab. 5). Of the national samples, 9% showed Al^{+3} contents that merit correction. As a province average, only Meta and Bolivar showed pH values in a lower range. However, Bolívar and the Santander municipalities of El Carmen and Rio Negro exhibited Al saturation % higher than 20%, suggesting that some type of amendment would be necessary (Tab. 2).

Ca saturation (% of ECEC) showed relationships similar to Al, which best explains the relationship with pH (R^2 = 0.75 vs. 0.63, respectively); however, it continues to be predictive beyond pH 5.5 (Fig. 8).

For cacao cultivated soils with a pH >5.0, Ca levels or its % saturation were at least 4.0 $cmol_c\ kg^{-1}$ or 60% saturation, respectively. These levels are proposed as indicators of the minimum soil Ca content or saturation for cacao soils in Colombia (Tab. 5), which corresponds to the lower limit of the levels proposed for other countries (Tab. 3). However, with the available Mg levels or its saturation no correlation was found with pH or other soil parameters (R^2 = 0.29 vs. 0.27, respectively) (Fig. 8). But the addition of Mg to the Ca contents relationship (Ca + Mg) did provide a greater precision. The suggested levels of available Mg for cacao according to the literature (Tab. 3) varied between 0.25 and 1.0 $cmol_c\ kg^{-1}$. For the Colombian soils, an Mg content of 1.0 $cmol_c\ kg^{-1}$, equivalent to 16% Mg saturation (Tab. 5) implies that 74% of Colombian soils show Mg deficiency. We interpret this level to be a higher limit to be used for soils with pH>5.5 and high ECEC. However, a lower limit of 0.50 $cmol_c\ kg^{-1}$ is suggested to be used for soil with a pH<5.5 (and lower ECEC) and equivalent to approximately 10% Mg saturation. From the available Mg requirements (Tab. 3), only Paranamantan (2000) proposed a lower level of 0.25 $cmol_c\ kg^{-1}$. The Mg requirements of productive cacao soils need to be an area of active research in Colombia.

The Ca/Mg ratio did not show any correlation with any chemical soil parameter (Fig. 9). The literature review does not report this parameter as an important indicator for cacao (Hartemink, 2005; Snoeck *et al*., 2016). Thirteen percent of the country's samples have a wide Ca/Mg ratio of >10 (Fig. 8). These soils are found mainly in the municipalities of El Carmen, San Vicente and Landazuri, all located in Santander. No apparent production problems are reported at these sites.

For available K, the range was between 0.002 and 1.9 $cmol_c\ kg^{-1}$ with a national average of 0.2 $cmol_c\ kg^{-1}$. The province range varied between 0.13 and 0.35 $cmol_c\ kg^{-1}$. No correlation was found between the content of available K and pH (R^2=0.0304) or another physicochemical soil parameter (Fig. 10). The level of 0.20 $cmol_c\ kg^{-1}$ for Colombia, equivalent to about 5% of the base saturation, is suggested as suitable for cacao (Tab. 5). These levels are similar to those suggested in other studies (Tab. 3). At this level, 70% of the Colombian cacao samples appear to have some level of deficiency.

Cation exchange capacity (CEC, measured at pH 7.0) was not evaluated, but the effective cation exchange capacity (ECEC) which is the sum of cations at soil pH is proposed

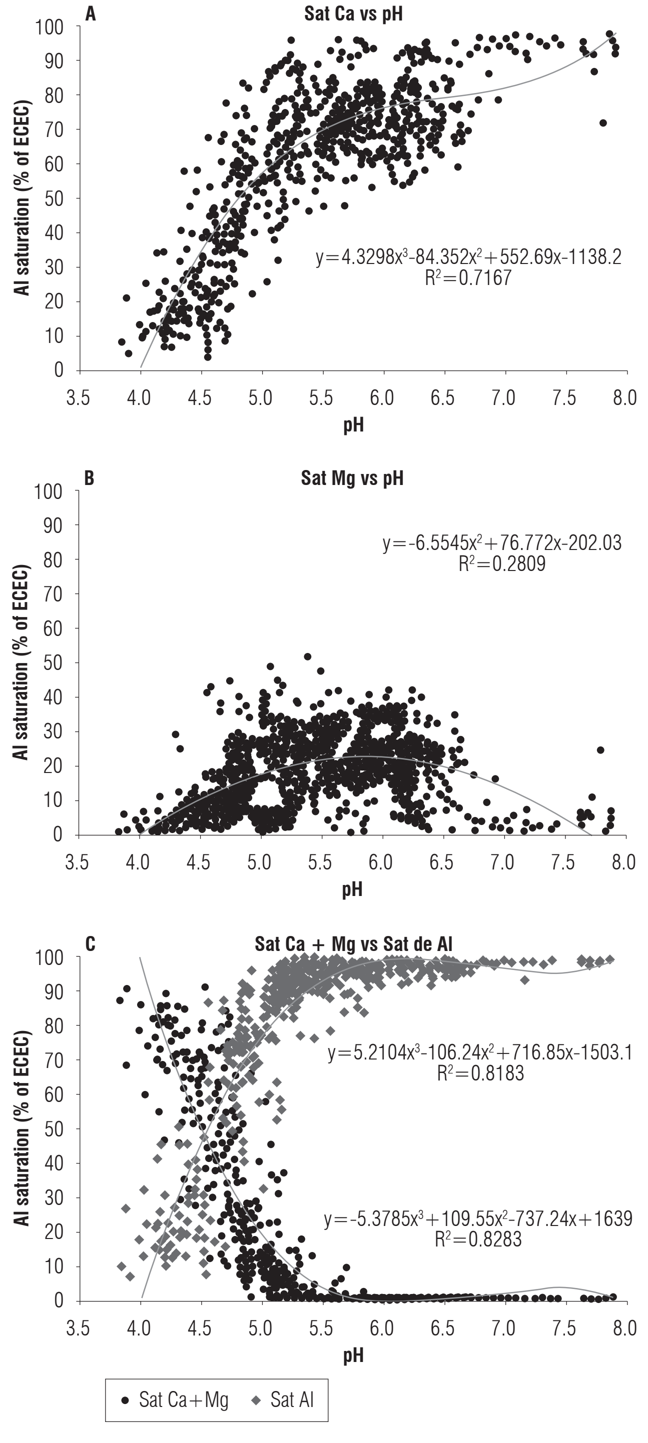

FIGURE 8. Relationship between pH: A) Ca saturation; B) Mg saturation; C) Ca+Mg saturation vs. Al saturation under representative cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013.

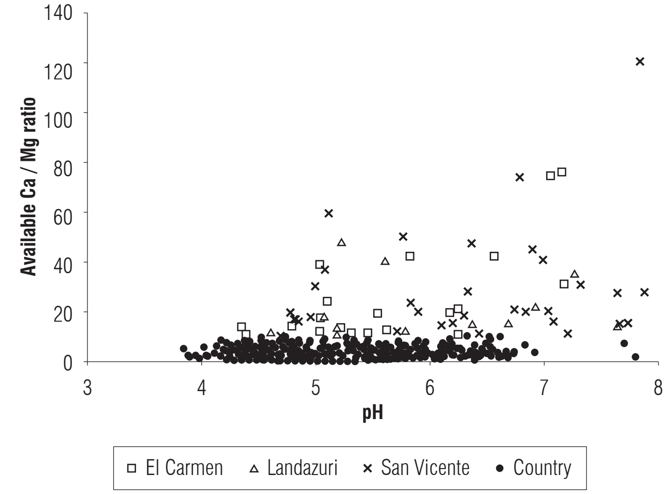

FIGURE 9. Ca/Mg ratio in cacao soils in Colombia from 2011 to 2013.

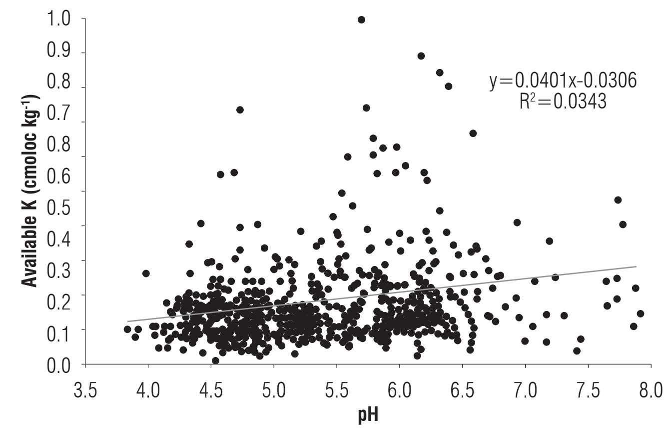

FIGURE 10. Available K content in the pH range of soils under representative cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013.

as a proxy indicator of the capacity to supply bases. ECEC was considered since it varies depending on the type and percentage of clay, the pH and the content of organic matter; and can hardly be changed permanently (Brown and Lemon, 2007). ECEC is proposed as a good indicator since cacao production is demanding on bases. The provinces ECEC range varied between 4.7 and 16.7 $cmol_c\ kg^{-1}$. The national range was between 1.1 and 33.2 $cmol_c\ kg^{-1}$ with a national average of 8.8 $cmol_c\ kg^{-1}$. A minimum level of 6.0 $cmol_c\ kg^{-1}$ is proposed for Colombia (Fig. 11), a much lower level than the proposed ones for other countries.

Conclusions

Based on the national survey carried out in this study, minimum physicochemical soil variables levels for productive cacao projects are proposed in Table 5. Under prevailing conditions, conditions, small cacao farmers of Colombia have a high probability of having at least two nutritional

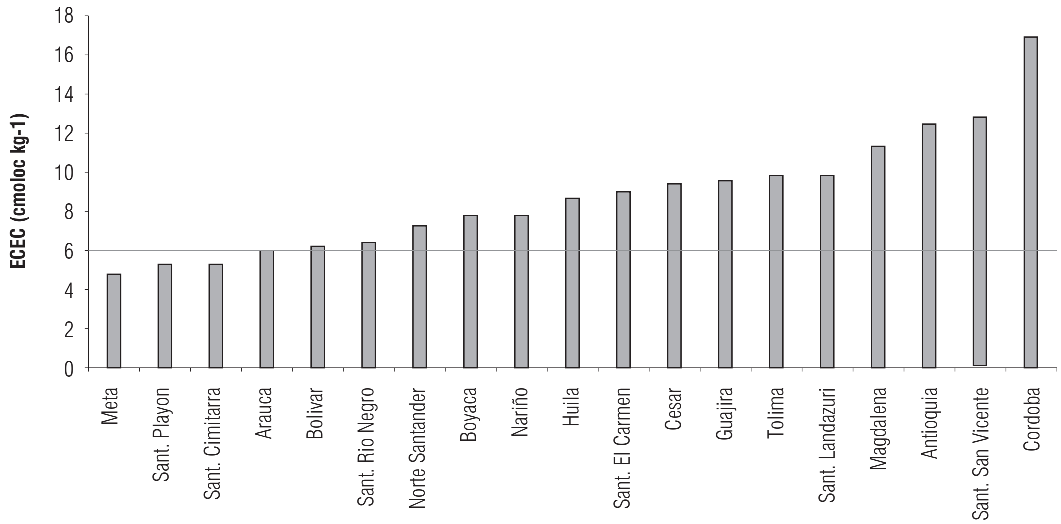

FIGURE 11. Average effective cation exchange capacity (ECEC) for representative cacao productive areas cultivated by small-scale farmers in Colombia from 2011 to 2013.

TABLE 5. Minimum levels of chemical conditions in Colombian soils for the development of productive cacao projects.

pH	SOM	P	Al	Ca	Mg	K	ECEC	Saturation			
								Al	Ca	Mg	K
	%	μg g^{-1}			cmol$_c$ kg^{-1}				%		
5.0	3.0	12	<1.5	4.0	1.0	0.2	6.0	<20	>60	>20	>5

deficiencies: P and K, and their correction is essential to increase productivity.

These suggested minimum levels are benchmarks for assessing the needs of an established crop or for starting new ventures in which medium to high productivity yields are expected: those that can produce sustainably at least 1.2 to 1.5 t ha^{-1}, which is the national goal for Colombia, are used as reference to apply for bank loans for successful productive projects (García-Cáceres *et al*., 2014). Of course, other additional factors must also be considered, such as environmental conditions and other soil conditions such as the water table and the absence of waterlogging conditions, as well as the proximity to collection centers.

With these minimum levels, plus an adequate agronomic fertilization management and sanitary control, it is possible to develop a productive and competitive cacao production for the social development of the country.

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Phosphate fertilization on soils with improved fertility in the Brazilian Cerrado

Fertilización fosfatada en suelos con fertilidad mejorada en el Cerrado brasileño

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ABSTRACT

The practices of soil amendment and fertilization carried out over the years have allowed the improvement of the fertility in some of them in the Brazilian soils. The objective of this study was to evaluate the performance of soybean in response to phosphate fertilization in soil with improved fertility. The research was carried out during the 2015-2016 harvest season in a dry-land area in the municipality of São Desidério, Bahia. The experiment was arranged in randomized blocks, with five treatments and four replicates. Treatments were composed by doses of phosphate fertilizer (0, 70, 140, 210 and 280 kg P_2O_5 ha^{-1}). The following variables were evaluated: plant height, height of insertion of the first pod, number of stems per plant, number of pods per plant, thousand grain weight, productivity, and the analysis of macro and micronutrients in the leaf. Soybean plants did not respond to phosphorus applied to soil with built fertility. Under the conditions of this study, phosphorus fertilization in soil with improved fertility did not promote productivity. Although the plants did not respond to foliar macro and micronutrients as a function of phosphate fertilization, there was no reduction of these in response to high doses.

Key words: adsorption of phosphorus, *Glycine max* L., foliar content.

RESUMEN

Las prácticas de corrección y fertilización realizadas a lo largo de los años en los suelos brasileños permitieron mejorar la fertilidad en algunos de ellos. El objetivo de este trabajo fue evaluar el desempeño de la soja en respuesta a la fertilización fosfatada en suelo con fertilidad mejorada. El trabajo fue realizado durante el cultivo 2015-2016 en el municipio de São Desidério, en Bahía. Se empleó un diseño experimental en bloques al azar, con cinco tratamientos y cuatro repeticiones. Los tratamientos fueron compuestos por dosis de fertilizante fosfatado (0, 70, 140, 210 y 280 kg ha^{-1} de P_2O_5). Se evaluaron las siguientes variables: altura de la planta, altura de inserción de la primera vaina, número de ramos por planta, número de vainas por planta, peso de mil granos, productividad y análisis de macro y micronutrientes en la hoja. Las plantas de soja no respondieron al fósforo aplicado en el suelo con fertilidad mejorada. En las condiciones de este trabajo, la fertilización fosfatada en suelo con fertilidad mejorada no promovió ganancia de productividad. Aunque las plantas no respondieron a los macro y micronutrientes foliares en función de la fertilización fosfatada, no hubo reducción de estos niveles en respuesta a las dosis elevadas.

Palabras clave: adsorción de fósforo, *Glycine max* L., contenido foliar.

Introduction

Soybean (*Glycine max* L.) is one of the main crops worldwide and recently one of the most growing productive crops in Brazil (Conab, 2018). The Cerrado biome, considered a region of low natural fertility and economic value until 1970, is currently the main soy producing region in the country (Leite *et al*., 2017; Trabaquini *et al*., 2017).

The outstanding results of soybean cultivation in the Brazilian Cerrado are due to the government incentives, genetic improvement and application of amendments and fertilizers; since these soils are mostly highly weathered and characterized by a low nutrient availability for the plants (Trabaquini *et al.,* 2017; Cattelan and Dall'Agnol, 2018). Among the essential plant nutrients, phosphorus is the element most commonly applied to the soil, because it is the nutrient that most limits the production in this region (Alcântara Neto *et al*., 2010).

The knowledge about the chemical limitations in Brazilian Cerrado soils, such as fixation in mineral colloids and by specific adsorption of phosphorus to Fe and Al oxides (Roy *et al*., 2017), made phosphate fertilization a routine for producers in this region. However, the execution of this practice over the years, along with the management and fertilization of other necessary nutrients, have made possible to modify the chemical, physical and biological

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properties of the soil, promoting the construction of soil fertility (Lacerda *et al*., 2015).

Santos *et al*. (2015) mentioned the fact that plants, such as soybean, present the potential to reuse the residual nutrients from previous fertilizations, thus, presenting low response to phosphate fertilization throughout the years of cultivation. Along with these factors, the excess of certain nutrients can affect the growth and productivity of the plants (Trabaquini *et al*., 2017).

Facing the changes in most of the soils of the Brazilian Cerrado over the years and the fact that producers continue the application of high doses of phosphate fertilizers, the objective of this study was to evaluate the performance of soybean in response to phosphate fertilization in soils with improved fertility.

Materials and methods

The study was carried out during the 2015-2016 harvest season in a dry land area in the municipality of São Desidério, Bahia (12°40’02” S and 45°57’47” W). The climate of the region is type Aw (hot and humid), according to the Köppen International Classification (Alvares *et al*., 2013). The soils of the experimental area present a Sandy Loam texture (Tab. 1), and according to the soil taxonomy keys are classified as Oxisols.

The experiment was arranged in randomized blocks, with five treatments and four replicates. The treatments were composed of different doses of phosphate fertilizer (0, 70, 140, 210 and 280 kg ha^{-1} of P_2O_5). The fertilization was carried out according to each treatment, with application in the planting row. Micronutrients were applied to the soil at the moment of sowing.

The sowing was carried out on November 11, 2015, with a mechanical seeder in an area under conventional planting system and using the cultivar M8349 IPRO®. The plant emergency occurred on December 4, 2015, with a final population of approximately 130,000 plants ha^{-1}. Temperature and precipitation data were collected during the experimental period (Fig. 1).

TABLE 1. Chemical and physical soil analysis of the experimental area at 0-20 cm depth.

pH ($CaCl_2$)	6.3	Potential acidity (H+Al) (cmol$_c$ kg^{-1})	1.0
Organic matter (g kg^{-1})	1.8	Sum of bases (Ca+Mg+K) (cmol$_c$ kg^{-1})	3.8
Available P (mg kg^{-1})	57.3	Cation exchange capacity (cmol$_c$ kg^{-1})	4.81
Available K (mg kg^{-1})	160.3	Base saturation (%)	79.2
Exchangeable Ca (cmol$_c$ kg^{-1})	2.6	Sand (%)	81.2
Exchangeable Mg (cmol$_c$ kg^{-1})	0.8	Silt (%)	1.3
Exchangeable Al (cmol$_c$ kg^{-1})	0.0	Clay (%)	17.5

Available phosphorus (P) and potassium (K) extraction with Mehlich-1; exchangeable calcium (Ca), magnesium (Mg) and aluminum (Al) extraction with KCl; H + Al extraction with calcium acetate.

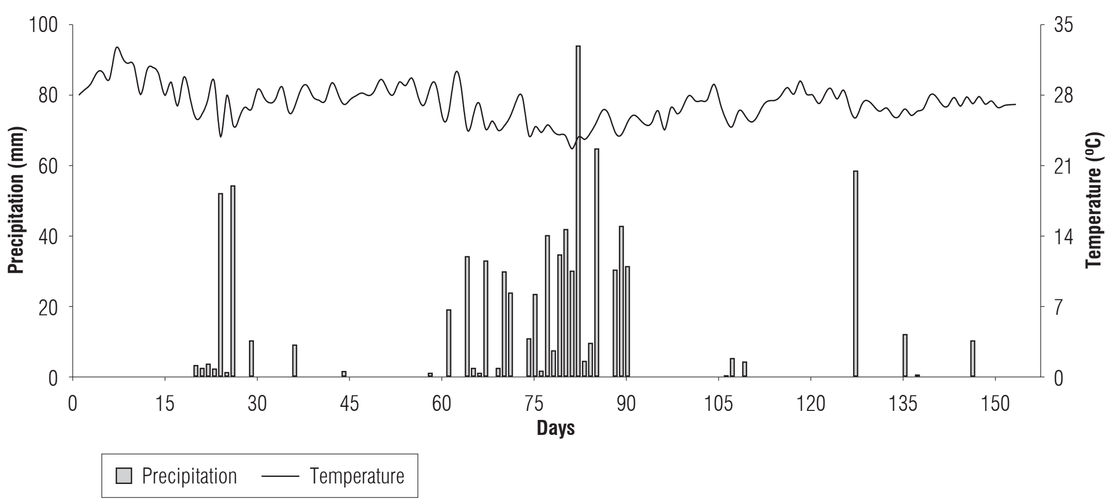

FIGURE 1. Average daily temperature and precipitation during the experiment.

Cultural management practices were performed according to the recommended baselines for soybeans in the Cerrado (Lopes, 2013). Weeds, pests and diseases were managed through applications of herbicides, insecticides and fungicides registered for the crop. During the phenological stages V3, V7 and R1 micro foliar Manganese was applied. The harvest was performed manually on January 4, 2016, removing 3 replicates of 2 representative linear meters of the plot, excluding the borders.

The following variables were evaluated: Plant height (HP) and height of insertion of the first pod (HIFP) were determined through direct measurement using a ruler graduated in millimeters. The number of stems per plant (NSP) and number of pods per plant (NPP) were determined through the manual counting of these indicators in the sampled plants. The weight of a thousand grains (WTG) was determined on a precision scale. The productivity (PD) was determined based on the grain yield of the plants harvested in each experiment and adjusted to 13% humidity, being corrected from the spacing and quantity of plants per linear meter and transformed to kg ha^{-1}. During the reproductive stage R1 (flowering plants), the third leaf was collected from the main apex of approximately 30 plants per treatment (Sousa and Lobato, 2004). Leaves were then packaged in paper bags identified according to the treatment and sent to the Laboratory of Plant Analysis of the Federal University of Tocantins for the determination of the macro and micronutrients content in leaves.

Initially, the data were tested for normality (Shapiro-Wilk) and homoscedasticity (Levene) and subsequently submitted to regression analysis, evaluating the significance of the betas and the determination coefficients to obtain the appropriate regression model, adopting a 5% probability.

Results and discussion

Among the soybean yield variables, only the first pod insertion height was adjusted to the regression model (Fig. 2). The height of plants did not present an adjustment to the regression model, producing plants with an average height of 64 cm, regardless of the applied phosphate dose.

The HIFP presented a linear reduction as a function of the increasing doses of P_2O_5 (Fig. 2B). For each kg of P_2O_5 applied to the soil, the plants showed a reduction of 0.0018 cm in HIFP. Despite the results, a low amplitude of variation in the plant HIFP (6.76 to 6.26) was observed as a function of the applied doses.

The number of stems presented a similar behavior to plant height with no adjustment to the regression model (Fig. 2C); plants had a mean number of stems around 12.68.

The height of plants and the number of stems presented a positive correlation with grain yield, since they provided a greater number of reproductive structures (Schoninger *et al*., 2015). The number of pods and the weight of the grains were also directly related to soybean yield. These variables were not altered as a function of phosphate fertilization in soils with improved fertility (Fig. 2). The height of insertion of the first pod had a direct influence on the occurrence of impurities and grain loss. Although it presented a linear reduction as a function of the doses of phosphorus (Fig. 2B), there was a low amplitude of variation (0.5 cm).

The soybean plants showed an average of 87.93 pods per soybean plant, regardless the phosphate dose (Fig. 2D). The non-adjustment of this variable to the regression model evidences the non-influence of phosphate doses on soils with good fertility.

Like the previous variables, the weight of a thousand grains did not fit the regression model (Fig. 2E) with plants presenting an average WTG of 130 g.

As expected, since the productivity indicators showed no adjustment to regression (Fig. 2), the productivity of soybean plants cultivated in soil with good phosphorus fertility did not present an adjustment to the proposed model (Fig. 2F). The average yield of the plants was 3033 kg ha^{-1}, independent of the phosphate dose applied to the soil.

When evaluating soybean yield in response to phosphate fertilization in Cerrado soils with high phosphorus availability, Santos *et al.* (2015) found an increase in productivity as a function of the applied doses. However, the increase in productivity (6%) is low in comparison to the high applied dose (389 kg ha^{-1} of P_2O_5), not being a viable practice as recommended by Lacerda *et al.* (2015).

In accordance with the results of this work, Lacerda *et al.* (2015) studied corn and soybean cultivation in Cerrado soils with high fertility and concluded that it is possible to grow soybeans for three consecutive crops without the use of phosphate fertilization and maintaining P levels in the soil. These authors carried out studies in a consolidated agricultural region in the country, but our study evaluated the behavior in the region denominated as the most recent Brazilian agricultural frontier (Leite *et al*., 2017).

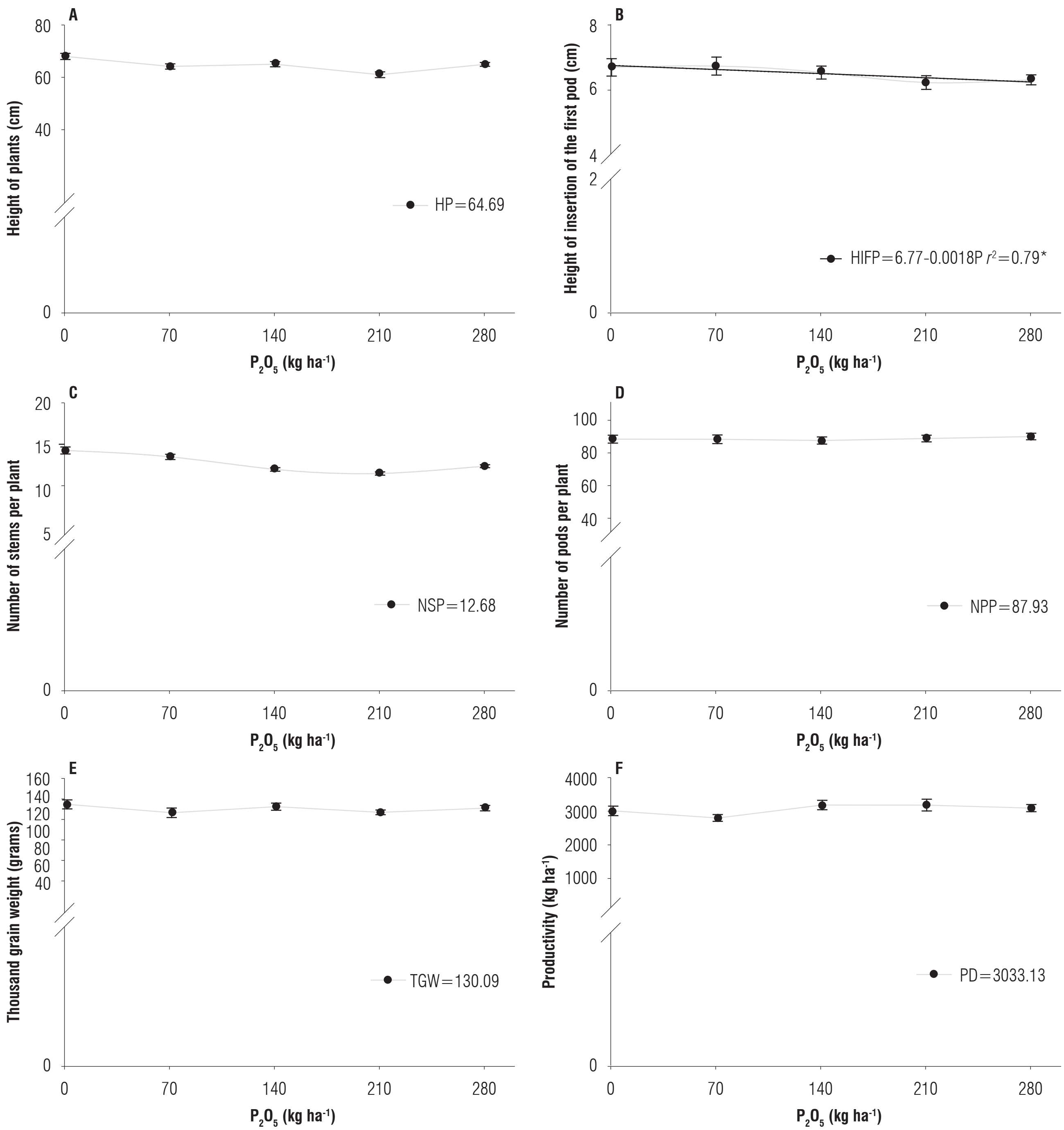

FIGURE 2. Height of plants (A), height of insertion of the first pod (B), number of stems per plant (C), number of pods per plant (D), thousand grain weight (E) and productivity (F) of soybean in response to phosphate fertilization in soil with improved fertility. $P \leq 0.05$.

Regarding the foliar levels of macronutrients of plants as a function of phosphate fertilization, only the levels of nitrogen and calcium were adjusted to the regression analysis (Fig. 3). All leaf mean values were compared to the minimum concentration levels for nutrients in soybean plants grown in the Brazilian Cerrado (Sousa and Lobato, 2004). The plants without phosphorus application presented an average content of 52.56 g kg^{-1} of N. From these values, the plants fertilized with phosphorus presented an increase of 0.017 g kg^{-1} of N for each kg of P_2O_5 applied to the soil. All doses presented leaf N levels above the reference level.

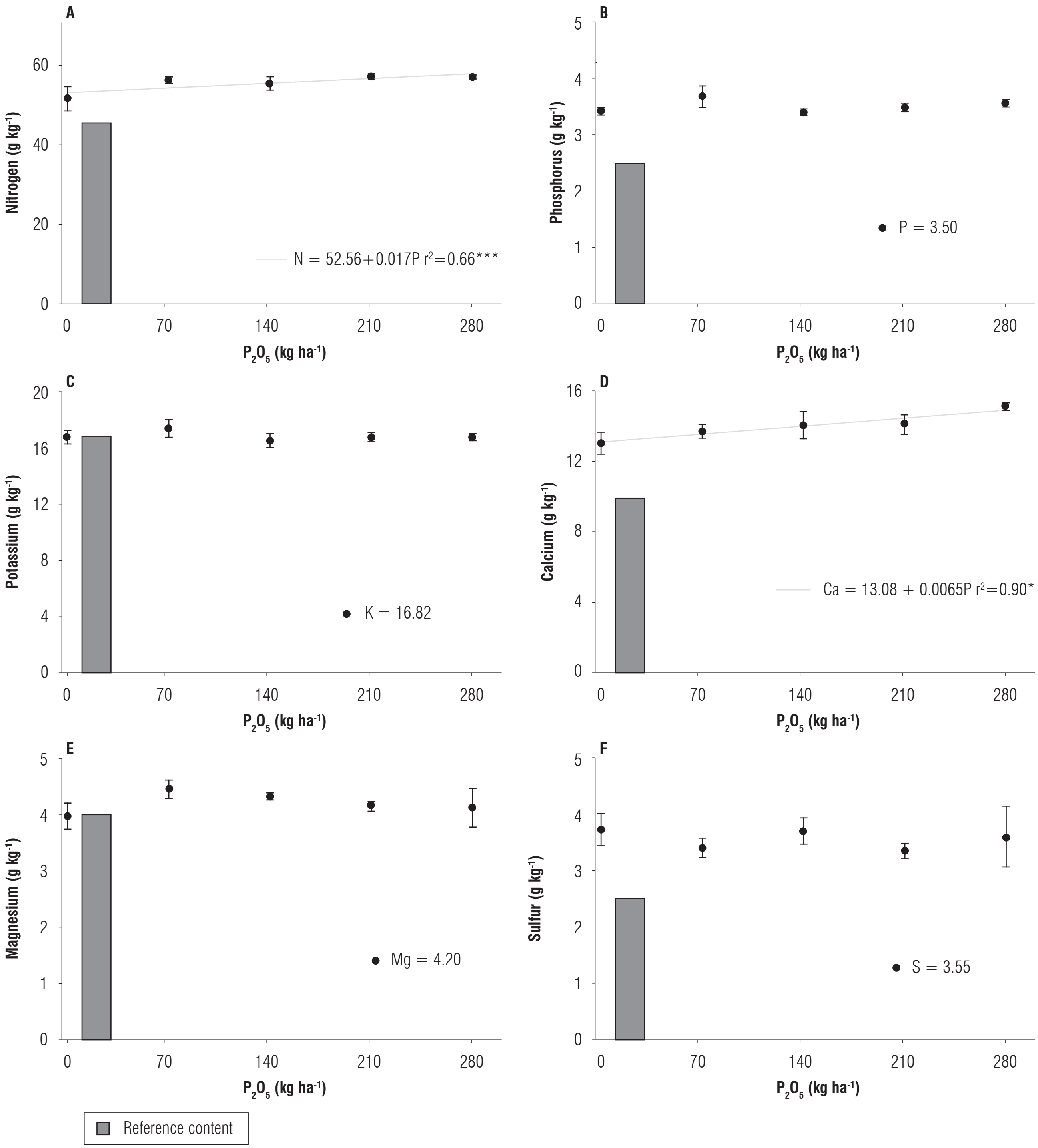

FIGURE 3. Leaf macronutrient contents: nitrogen (A), phosphorus (B), potassium (C), calcium (D), magnesium (E) and sulfur (F) in soybean plants with phosphate fertilization.

The average levels of leaf phosphorus did not adjust to the regression model as a function of the fertilizer applied to the soil (Fig. 3B). The results showed that there is no greater accumulation of this nutrient in the plant due to the increase of the doses in soil with good fertility.

For the content of foliar potassium, there was no adjustment to the regression as a function of the phosphorus doses applied (Fig. 3C). The mean leaf potassium content was 16.82, which ranged in the appropriate levels for soybean.

Regarding the foliar calcium contents in response to the phosphate doses, they presented an adjustment to the linear model increase (Fig. 3D). For each kg of P_2O_5 applied to the soil, the plants had an increase of 0.0065 g kg^{-1} Ca in the leaf. Despite the increase in Ca, even plants without fertilizer application presented a foliar Ca level above the suitable level for soybean cultivation in the Brazilian Cerrado.

Magnesium and sulfur leaf contents showed no adjustment to the linear regression (Fig. 3E), producing plants with average leaf contents of 4.20 and 3.55 g kg^{-1}, respectively. For these two nutrients, the plants presented average levels above the appropriate level. In the foliar micronutrient contents, only the manganese was adjusted to the regression (Fig. 4). Leaf boron content in the plants was 37.90

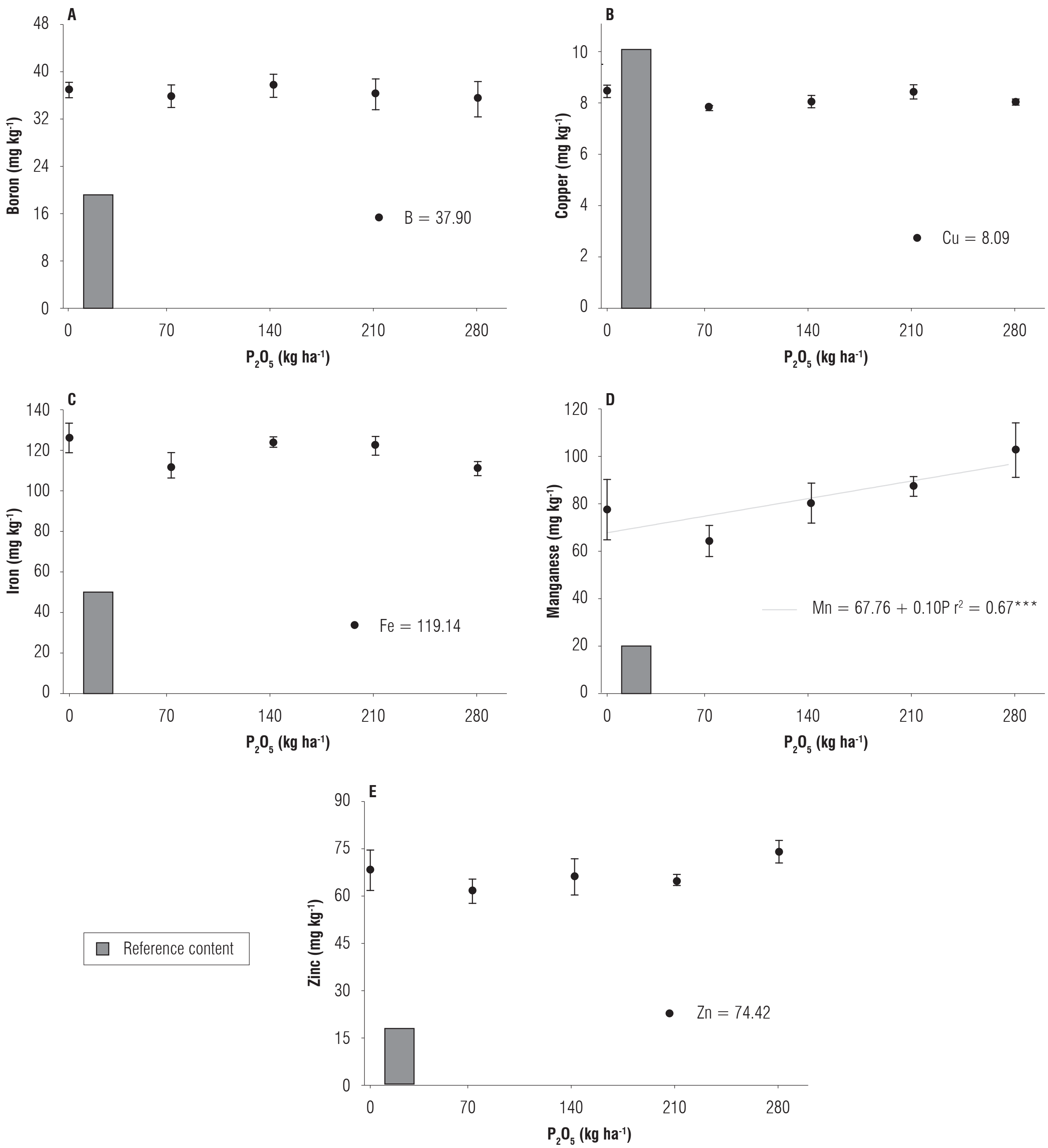

FIGURE 4. Leaf micronutrient contents: boron (A), copper (B), iron (C), manganese (D) and zinc (E) in soybean plants with phosphate fertilization.

mg kg^{-1} (Fig. 4D), being this value above the appropriate minimum level.

As in the case of boron, copper and iron contents did not adjust to the regression (Fig. 4B and C). However, the leaf content of copper was below the appropriate minimum level for the crop which was different from the behavior of all other foliar nutrients (Fig. 4B). Iron content in the leaf was above the appropriate minimum level at all doses evaluated.

Copper contents in the leaf were below adequate levels at all doses evaluated (Fig. 4B). The average copper leaf content found (8.09 mg kg^{-1}) ranged below the levels of soybean plants from high productivity crops in Brazil and the United States (Mascarenhas *et al*., 2013). The deficiency of this micronutrient causes a reduction in the growth of soybean plants (Bruns, 2017), directly affecting the yield of the crop (Dimkpa *et al*., 2017). Although agricultural pesticides commonly used in soybeans, mainly fungicides, have copper in their formulations (Bruns, 2017), Brazilian Cerrado soils are deficient in this nutrient (Marques *et al*., 2004).

Foliar manganese was 67.76 mg kg^{-1} in the absence of phosphate fertilizer; however, with the fertilizer application, the plants presented an increase of 0.1 mg kg^{-1} of Mn to each kg of P_2O_5 applied to the soil (Fig. 4D).

Regarding zinc, although there was no adjustment to regression, leaf contents of these micronutrients were verified above the minimum suitable level for soybean cultivation at all doses (Fig. 4E).

Evaluating the ideal levels of nutrients for the soybean cultivation in the Brazilian Cerrado (Sousa and Lobato, 2004), the studied soil has an adequate fertility for cultivation (Tab. 1), which reinforces the idea of soil with improved fertility. Such high fertility in these soils occurs because Brazilian agricultural fields received more phosphate fertilizers than necessary for the need of crops, a practice that has been carried out since 1970 (Roy *et al*., 2017; Withers *et al*., 2018) and that has consequently accumulated large reserves of phosphorus in the soil.

This research is innovative, since besides studying the productivity of soybean under phosphate fertilization, we also studied the possibility of translocation and nutrient uptake problems in plants due to the high level of phosphorus in the soil associated with doses of phosphate fertilizer. However, even at the highest phosphate doses studied, the plants did not present deficiency of macro and foliar micronutrients except for copper (Figs. 3 and 4). These results are important because micronutrient deficiency can significantly reduce plant quality and productivity (Sutradhar *et al*., 2017).

It is known that global phosphorus reserves are a finite and critical natural resource. Since Brazilian phosphate rock mines provide limited amounts of phosphorus, phosphate fertilizers are mostly imported, which makes Brazilian agriculture vulnerable to future phosphorous shortage and sudden price fluctuations (Withers *et al*., 2018).

In order to consider Brazilian agricultural systems as sustainable in the future, it is necessary to use phosphate fertilizers more efficiently (Withers *et al*., 2018). When considering the amount of phosphorus immobilized in the plant grains (Sousa and Lobato, 2004) the level of phosphorus in the studied soil (Tab. 1) would enable the cultivation of soybeans during three consecutive harvests without the practice of phosphate fertilization. This would allow obtaining the same productivity found (3033 kg ha^{-1}) and still maintaining high nutrient levels in soil. From the third harvest on, phosphorus should be applied in replacement to the immobilized amounts of this nutrient by the grains of the plants (5-8 kg t^{-1} of grain produced), to maintain the level of phosphorus in the soil.

Conclusions

It was observed that phosphate fertilization for soybean production was not necessary in soil with a high content of available phosphorus.

Despite the non-response to productivity, high doses of phosphate fertilization did not affect nutrient uptake in plants.

Under the conditions of this experiment, phosphorus fertilization in soil with improved fertility does not promote productivity.

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Effect of climate variability on *Collaria scenica* (Hemiptera: Miridae) on the Bogotá plateau

Efecto de la variabilidad climática sobre *Collaria scenica* (Hemiptera: Miridae) en la Sabana de Bogotá

Andrea Rodríguez-Roa[1*], Blanca Arce-Barboza[2], Francisco Boshell-Villamarin[3], and Nancy Barreto-Triana[1]

ABSTRACT

The aim of this research was to study the effect of climatic conditions on the population of the grass bug *Collaria scenica* (Stal, 1859) using agroclimatic models and analyzing its behavior under interannual variability scenarios related to El Niño-Southern Oscillation (ENSO) events. The population fluctuations of this grass bug were modeled, estimating significant climatic variables in the presence of nymphs and adults with a multiple linear regression analysis. The population distribution of this insect in relation to the occurrence of the El Niño and La Niña phenomena on the Bogota plateau was analyzed based on variations of the sea surface temperature (SST) in the tropical Pacific and their impact on climate variables. The maximum and minimum temperatures, precipitation and evapotranspiration showed higher significance for this pest than the other variables. The optimal growth and development conditions for this grass bug occurred during periods with a higher daily thermal amplitude and high precipitation values, which highlights the positive effect of abundant, but not excessive, rain. This study helped to determine the population growth during the two seasons of the year with higher rainfall in the area, which correspond to March-May (MAM) and September-November (SON), mainly in the following season after the dry quarter of December-February (DJF). Important increases occur in the El Niño event because of the greater accumulation of heat units during this phenomenon based on increases in air temperature that favor insect growth.

Key words: climatic variables, El Niño-Southern Oscillation, grass bug, models, pest.

RESUMEN

El objetivo de este trabajo fue estudiar el efecto de las condiciones climáticas sobre la población de la chinche de los pastos *Collaria scenica* (Stal, 1859) a partir de modelos agroclimáticos, y analizar su comportamiento bajo escenarios de variabilidad interanual, relacionados con eventos El Niño-Oscilación del Sur (ENOS). Para esto, se modeló la fluctuación poblacional de la chinche de los pastos *Collaria scenica* (Stal, 1859) estimando las variables climáticas significativas en la presencia de ninfas y adultos, a través de un modelo de regresión lineal múltiple. Se analizó la distribución poblacional del insecto asociada a la ocurrencia de los fenómenos de El Niño y La Niña en la Sabana de Bogotá, tomando como base la variación de la temperatura superficial del mar (TSM) en el Pacífico tropical, y su impacto en las variables climáticas. La temperatura máxima y mínima, la precipitación y la evapotranspiración mostraron mayor significancia para la plaga frente a las demás variables. Las condiciones óptimas para el crecimiento y el desarrollo de la chinche se dan para periodos con mayor amplitud térmica diaria y valores altos de precipitación, destacando el efecto positivo de las lluvias abundantes, pero no excesivas. El estudio permitió establecer aumentos de la población durante las dos épocas del año con mayor lluvia en la zona, correspondientes a marzo-mayo (MAM) y septiembre-noviembre (SON), principalmente en la temporada siguiente al trimestre seco de diciembre-febrero (DEF). Se presentan incrementos importantes bajo un evento El Niño, como consecuencia de la mayor acumulación de unidades de calor que se origina durante este fenómeno, a partir del aumento de la temperatura del aire que favorece el crecimiento del insecto.

Palabras clave: modelos, plagas, chinche de los pastos, variables climáticas, El Niño-Oscilación del Sur.

Introduction

One of the main economic activities on the Bogota plateau is specialized dairy production because this region has suitable environmental conditions for its development. These production systems are thriving on the Bogota plateau because of its particular climatic (i.e. average temperature of 13°C, altitude between 2,500 and 3,000 m a.s.l., 70% relative humidity and a bimodal precipitation regime) and edaphic conditions. Another factor that has contributed

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to the development of this industry is the availability of kikuyu grass (*Cenchrus clandestinus* Hochst. ex Chiov, Morrones synonym of *Pennisetum clandestinum* Hochst. ex Chiov) and ryegrass (*Lolium* spp.), forage used for grazing specialized dairy cow breeds (Benavides, 1997; Duarte *et al*., 1998; Martínez and Barreto, 1998).

In these specialized dairy production systems, a reduction in milk production is a consequence of the presence of pests such as the grass bug *Collaria scenica* (Stal, 1859), an insect that sucks sap from the leaves of grasses, causing different degrees of damage and generating tissue death that further causes a reduction in biomass availability and affects forage quality. This leads to an income reduction of close to 25% for producers (Duarte *et al*., 1998; Martínez and Barreto, 1998).

This pest has been recorded on the Bogota plateau since 1988 and has expanded to other milk-producing regions, such as the Ubate and Chiquinquira valleys and Alto Chicamocha (Duarte *et al*., 1998; Ramírez and Díaz, 2002; Ferreira *et al*., 2013).

Insect development and behavior are affected by interactions with the environment, including climatic variables such as temperature, precipitation, relative humidity and wind, among others. According to Boshell (2010), the distribution of insects and the duration of their life cycle are affected by climatic conditions, especially temperature. Therefore, knowledge on the natural climatic variation of an area and its impact on insects or pests is important since this constitutes a significant issue for the prevention of sanitary problems.

Climate variability and climate change have potential impacts on agriculture and particularly on pest behavior and development. Climate variability refers to variations in the mean state and other climate statistics (standard deviations or occurrence of extremes, etc.) on all temporal and spatial scales beyond those of individual weather events. Climate change refers to any change in climate over time, whether as a result of natural variability or anthropogenic forces (IPCC, 2014).

The Bogota plateau is continually affected by climatic anomalies. The agroclimatic quarters or seasons with lower rainfall correspond to the months of December-February (DJF) and June-August (JJA), and the periods with higher rainfall are March-May (MAM) and September-November (SON), all of which are altered by large-scale climatic phenomena such as El Niño-Southern Oscillation (ENSO) events, which generate drier or rainier periods (Pabón and Torres, 2006). These events cause disasters in a large part of the region, and the presence of El Niño has serious consequences as a result of precipitation deficits and increases in temperature and frost threat. The more obvious agricultural and socioeconomic impacts include decreases in the production of basic crops, increased incidence of pests and diseases, reduction of river and stream levels, economic losses, and high product prices, among others.

Studies on climate variability and global climate change have shown that future variations in climate will generate a noticeable effect on the migration and abundance of pest insect populations, which will adversely affect global agricultural and livestock productivity (Palikhe, 2007; Merril *et al*., 2008). An accelerated change in the spatial distribution range of insect species will be one of the important effects of climate variability and change (Boshell, 2010). As the planet heats up, insect species limited by temperature will be able to expand to other areas as fast as their own dispersion mechanisms allow them. For example, in the case of the Miridae family (i.e. the family grass bugs belong to), an increase in temperature would work in their favor more than other grass pests (Kiritani, 2006).

For example, variations resulting from climate change affect the potato moth *Phthorimaea operculella* (Zeller). According to Kroschel *et al.* (2013), the potential for damage will gradually grow in all regions where this pest currently exists, with a significant increase in the tropics and subtropics in warmer regions where potatoes are grown under emissions scenarios SRES (Special Report on Emissions Scenarios) -A1B (2050), which model future climatic conditions with a balanced emphasis on all energy sources. Also, an increase in temperature in more temperate climate regions, such as mountainous areas and inter-Andean valleys, will generate a moderate increase in damage potential in new areas with an infestation risk.

According to Pulido (2016), for populations of the common cattle tick *Rhipicephalus (Boophilus) microplus* in the Cundiboyacense highland area, it might be possible that climate change will not have a positive influence on this species since there is a decrease in all the stages of development when including data for climatic projections for the period 2011-2040 according to the climate change scenarios of the Third National Communication (IDEAM *et al*., 2015).

In a study by Ramirez *et al.* (2015), nonparametric trend analysis (Mann-Kendall) and correlation analysis were used to determine the effects of climate variability associated with the occurrence of El Niño and La Niña on the thermal time of the coffee berry borer. This effect is differential: above 1,400 m a.s.l., El Niño increases and La Niña decreases the suitable conditions for this insect's growth and development, while below 1,400 m a.s.l., El Niño reduces and La Niña increases these conditions.

Additionally, Giraldo *et al.* (2011) reported that high temperatures during the El Niño phenomenon favor the reproduction of the coffee red spider mite *O. yothersi*.

A study carried out in the central coffee growing zone of Colombia linked three climatic periods (neutral period 2007, La Niña period 2008, and El Niño period 2009 - 2010) with the dynamics of coffee berry borer infestation. The results showed that, during the El Niño phenomenon, there were increases in the average air temperature and in the levels of infestation in comparison with the neutral and La Niña periods. In conclusion, temperature was the variable that had the greatest influence on the development of the coffee berry borer, accelerating its life cycle and, therefore, producing more progenies in less time (Constantino *et al.*, 2011).

According to Ramírez and Díaz (2002), in the case of grass bugs, population density has a direct relationship with altitude and temperature; the highest densities are found between 2,000 and 2,400 m a.s.l., whereas populations have very low densities above 2,700 m a.s.l. Barreto *et al.* (2011) found significant spatial and altitudinal pest increases in farms located between 1,600 and 3,000 m a.s.l. on the Bogota plateau, in the Ubate and Chiquinquira valleys, and in the Alto Chicamocha region.

According to the modeling of the current potential distribution of *C. scenica* and under the climate change scenario A1 (characterized by a low population trajectory and rapid economic growth) for the period of 2020 in the livestock areas of the Cundiboyacense highland region (Pulido *et al.*, 2011), there is an expansion of potential areas, both latitudinally and towards higher altitude areas; this expansion is mainly connected with grazing areas and associations of crops and forests, with more extensive areas for these two land uses. The potential areas with the highest probability of insect presence were found at elevations higher than 2,800 and even up to 3,000 m a.s.l. The model was validated in 2011 in the field on farms located between 2,800 and 3,400 m a.s.l., corroborating the presence of the pest.

Taking into account the importance of *C. scenica* in milk production on the Bogota plateau and given the fact that variability and climate change may favor the expansion and growth of its populations, it is important to value the relationship between climate and the abundance and distribution of this species. This study considers the application of mathematical and statistical models for the description of pest and disease behavior in agriculture as an important step towards a better understanding of the epidemiology of pests, and as an opportunity to support producers in the optimization of crop protection management (Orlandini *et al.*, 2017). Therefore, the aim of this research was to study the effect of climatic conditions on this grass bug's population using agroclimatological models and to analyze its behavior under scenarios of interannual variability related to ENSO events.

Materials and methods

Sampling

To register the presence of this insect, weekly sampling was conducted from May, 2009 to November, 2010 at a dairy farm located in Funza (part of the Bogota metropolitan area), in the Western Savanna Province, of the Department of Cundinamarca, (4°39'24.77" N and 74°11'41.60" W, at an altitude of 2,561 m a.s.l), where three enclosed pastures were selected with different resting periods (d) after grazing, as follows: one recently grazed, another halfway through the resting cycle, and the third one with grazing. In each plot, a quadrant of a quarter of a hectare was selected, where a sampling grid with 10 points was established. A sample was taken at each point, which consisted of ten double passes with an entomological net in a 10 m linear transect, for a total of 10 samples per pasture, according to the methodologies described by Barreto *et al.* (1996) and Garza and Barreto (2011). The dimensions of the entomological net were 40 cm in diameter and 90 cm in length, attached to a 120 cm handle. In addition, information on the pasture management practices that affected the pest population was also registered.

To establish the relationship between the grass bug and agroclimatic variables, the total number of fourth and fifth nymphal stage individuals captured and the total number of adults were analyzed. For the climatic information, daily precipitation, maximum and minimum temperatures, relative humidity, wind speed and solar brightness, data from the 2009-2010 period were used, retrieved from a meteorological station located at 4°41' N and 74°12' W, 2,543 m a.s.l. The daily climatic information was accumulated weekly to build the agroclimatic indices and was crossed with the number of weekly nymph and adult captures.

Multiple regression model

Regression models are used to describe the population's behavior over time, which identified the key factors for the populations and generated approximations for estimating growth (Boyce, 2002). To evaluate the behavior of this grass bug in relation to agroclimatic conditions, a multiple regression analysis was carried out. Variables that did not show a normal distribution were transformed using the box-cox method (Mendoza *et al*., 2002). The goodness of fit of the theoretical model was evaluated using the coefficient of determination, and the explanatory capacity was obtained from the least squares method (ANOVA), the result was evaluated using Fisher's F-value. The statistical significance of the model's variables on the dependent variable was carried out using a Student's T -test.

In the regression analysis, the calculation of the following agroclimatic indices was integrated in the following equation:

Degree day (*GD* or GDD) represents the accumulation of heat units or number of degree days above a certain lower limit temperature (Wang, 1960; Cross and Zuber, 1972; Russelle *et al*., 1984), which for the case of insects corresponds to the minimum development threshold (base temperature). For each day, the *GD* was calculated as:

$$GD = \left(\frac{T_{max} + T_{min}}{2} \right) - T_b \quad (1)$$

where:

GD = Degree days

T_{max} = Maximum daily temperature

T_{min} = Minimum daily temperature

T_b = Minimum threshold temperature at base temperature

In the developmental stage prediction from degree days, it was necessary to establish the T_b and the number of degree days that must be accumulated in each biological stage (egg, nymphs and adult, in the case of this grass bug) to continue with the next growth phase or termination of the cycle as an adult. According to Martínez and Barreto (1998), the average life cycle of grass bugs varies according to temperature conditions. In a laboratory with a constant temperature of 18°C, the time from egg to adults is 44 d, while in the field with an average temperature of 13°C, it is 65.5 d. Another study under insectary conditions with temperature fluctuation and two host plants (common oat *Avena sativa* and kikuyo *Pennisetum clandestinum*), showed that the egg stage has an incubation period of 30 d and the five nymphal instars had the following periods: I: 8-13.5 d; II: 4.5-9.5 d, III: 4-6.4 d, IV: 6-6.4 d, and V: 6.7-10.1 d; for an average duration from egg to adult of 65 d in oat and 70.7 d in Kikuyo (Barreto, 2012).

In this study, pest information was obtained on the Bogota plateau where there are optimal development conditions, and the average temperature is approximately 14 ± 1°C. In addition, since laboratory experiments have shown that this insect does not survive at constant temperatures below 12°C, a T_b of 13°C was considered (Barreto *et al*., 2012).

Degree days were calculated for the same period of time in which the population data were collected (May 2009 to November 2010) with daily T_{max} and T_{min} values.

Water index

Water balances evaluate water availability in a specific place and period, which serves as a basis to define different agricultural activities, such as crop establishment, monitoring and growth, definition of management activities, appearance of pests and diseases, and irrigation programming, among others. Water balance is determined by water inlets and outlets in a system. Contributions mainly come from precipitation, and losses are caused by evaporation and transpiration (evapotranspiration). Therefore, to establish water availability, these two variables can be compared by calculating either their difference (precipitation minus evapotranspiration) or their relationship (precipitation over evapotranspiration), which results in a series of values representing potential losses or gains from moisture stored in the soil (Jaramillo, 1982).

A water index was estimated when the relationship between the insect pest and the agroclimatic variables was established. This, in turn, estimated the water availability by comparing the precipitation and evapotranspiration using the following equation:

$$IH = \frac{PPT}{ETo} \quad (2)$$

where:

IH = Water index

PPT = Precipitation

ETo = Reference evapotranspiration, calculated through the Fao-Penman-Monteith method.

Note that, when *PPT* is higher than *ETo* (positive balance), the ratio is higher than one.

Model validation

The behavior of the population estimated with the regression model was validated by comparing it with the observed data with a correlation coefficient and the respective significance in the two cases: 1) observations of where the model comes from: comparison with observations obtained from the farm located in the municipality of Funza from May, 2009 to November, 2010 (data used to build the model) and 2) observations of where the model comes from in a later period: the main objective of the model is the prediction of the pest, so its response was evaluated with real values in a period after the one considered for its construction. Therefore, estimates for nymphs and adults were compared with data obtained from December, 2010 to March, 2011.

Interannual climatic variability analysis

In order to estimate the behavior of the grass bug population under climatic variability scenarios, information from the period 1980-2010 was used from seven meteorological stations distributed on the Bogota plateau (Tab. 1).

TABLE 1. Location of the meteorological stations used in this study.

Station code	Station name	Municipality	Altitude (m a.s.l.)	Latitude (°N)	Longitude (°W)
2401512	Isla Santuario	Fuquene	2,580	5.28	73.44
2120574	Silos	Choconta	2,709	5.07	73.42
2120572	Gja San Jorge	Soacha	2,900	4.30	74.11
2120570	Guasca	Guasca	2,750	4.52	73.52
2120598	Gja Providencia	Tenjo	2,560	4.47	74.12
2120542	Tibaitata	Mosquera	2,543	4.41	74.12
2120579	El Dorado	Bogota	2,547	4.42	74.09

The behavior of pest populations in relation to the conditions generated by the oceanic-atmospheric phenomenon ENSO on the Bogota plateau was estimated considering the agroclimatic seasons. The impact of the ENSO cycle on the study area was identified based on the spectral analysis of the series of anomalies shown by climatic variables that favor the appearance of the pest, recognizing the periodicity of the cycle in the series.

Then, variability scenarios were established with a correlation analysis between the anomalies of the climatic variables and the anomaly of the sea surface temperature (SST) of the tropical Pacific Ocean. These scenarios were constructed by grouping the quarters by El Niño and La Niña events and neutral years during the period 1980 to 2009 based on the oceanic Niño index (ONI) taken from the database of the Climate Prediction Center (CPC) of the National Centers for Environmental Prediction (NCEP) and the National Oceanic and Atmospheric Administration (NOAA). Once these periods were established, the average and standard deviations were obtained for each variable; then, the insect population in the region was estimated under each climatic variability scenario.

Results and discussion

The multiple regression model adjusted with grass bug nymph and adult population values resulted in the combination of the variables *GD* and *IH*, which were significant and could be related in the following equations:

$$(NymphsT)^{-2} = -0.1898 + 0.0008\,GD + 0.0066\,IH - 0.0012\,IH^2 \quad (3)$$

with R^2 = 14.5% for nymphs, and where:

$$NymphsT = e^{\,NymphsT} - 10 \quad (4)$$

and

$$(AdultsT)^{-2} = -0.745 + 0.0012GD + 0.0049\,IH - 0.0015\,IH^2 \quad (5)$$

with R^2 = 20.4% for adults, and where:

$$Adults = e^{\,AdultsT} - 10 \quad (6)$$

The obtained model is useful for predicting and indicating the distribution tendency of the grass bug population, and the above equations correspond to the best estimates of nymph and adult population fluctuations in relation to the analyzed agroclimatic conditions. This showed that the fluctuation behavior of nymphs (14.5%) and adults (20.4%) is explained by *GD* and *IH*. *GD* explains pest growth according to the accumulation of temperature, and *IH* explains the effect of water availability on the soil surface. These conditions may or may not favor both species development and grass growth since grass is the food of the pest and will attract either a higher or lower number of insects.

The coefficients of determination are considered statistically acceptable with high significance for a *P*-value of less than 0.05 in each of the factors of the equation. The *P*-value in the ANOVA table (Tab. 2) is less than 0.05, which indicates a significant relationship with a 95% confidence level. The remaining unexplained percentages are attributed to other non-agroclimatic factors, such as the quantity and temporary quality of the pasture as a food source, natural enemies, control of other insects, and the presence of other hosts, among others. Moreover, the way in which the insect data were obtained (i.e. field values) had some inherent problems that resulted in a decrease in the R^2 values.

TABLE 2. Analysis of variance.

Source	GL	SC Sec.	SC. Ajust.	CM Ajust	F-ratio	*P*-value
Nymphs model	4	0.033685	0.033685	0.0084212	9.19003	0.000001
Adults model	4	0.108589	0.108589	0.0271472	13.8573	$<$0.000001

The result of this model, in which *GD* has a positive contribution for the grass bug population, agrees with different organism growth models, where the index expressed in degree days establishes the average time in which a species has optimal development conditions, as explained by Ramírez *et al.* (2015). The model responds to the growth relationship between different pests and climatic variables, as in the cases of the mustard aphid *Lipaphis erysimi* (Kalt) (Narjary *et al.*, 2013) or the mango leafhopper *Idioscopus* spp (Gundappa *et al.*, 2018), in which a model to predict their population can be obtained from a regression analysis.

First validation

When comparing the nymph and adult fluctuation from the data taken in the field (in the absence of grazing and chemical control) and the values given by the regression model, the performance of the model in reproducing pest increases and decreases is evidenced for each date (Fig. 1 and Fig. 2). These values coincided for some peaks, but the simulation underestimated most of the relevant growth, especially in the case of the nymphs. Therefore, it can be inferred that the model is useful for determining the seasons in which the presence of the pest could increase or decrease under the predicted climatic conditions. The

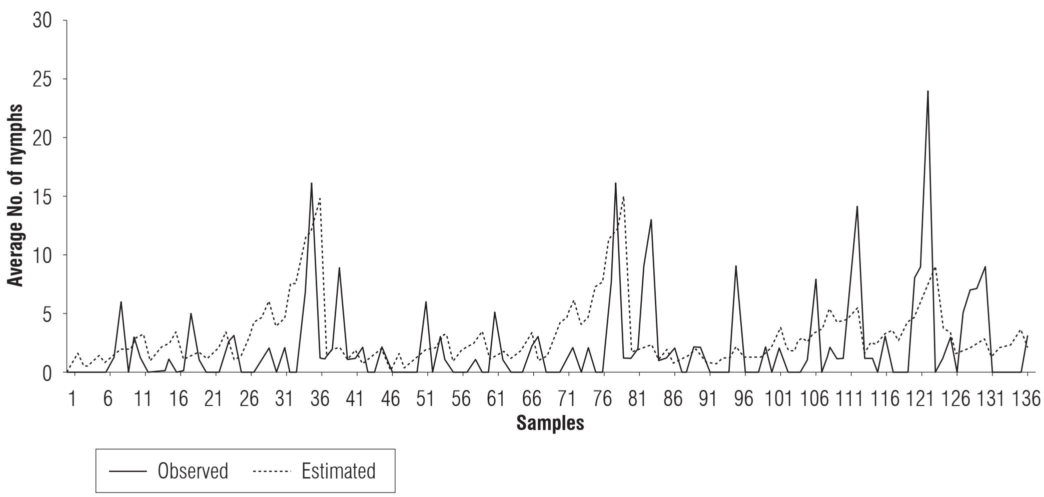

FIGURE 1. Comparison of population fluctuation observations and estimates of *Collaria scenica* nymphs from May, 2009 to November, 2010.

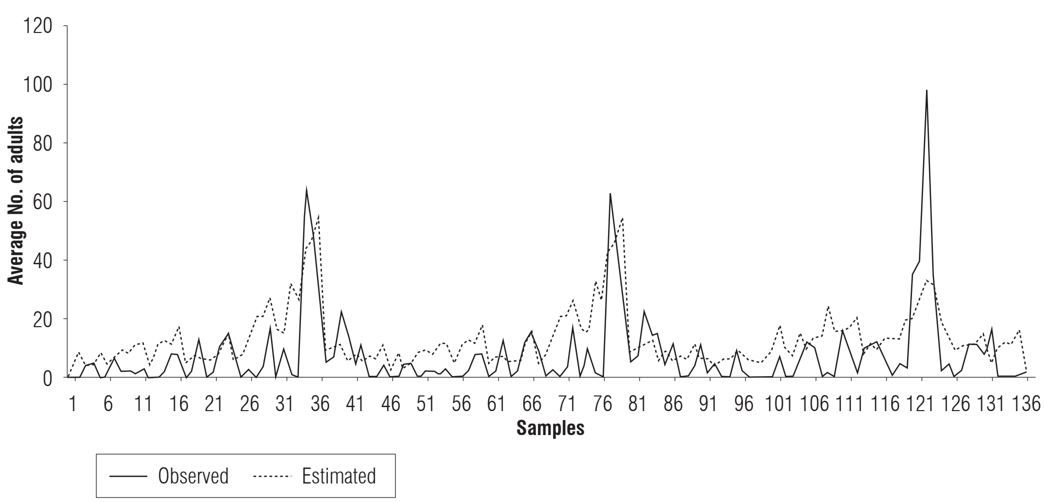

FIGURE 2. Comparison of population fluctuation observations and estimates of *Collaria scenica* adults from May, 2009 to November, 2010.

model shows a gradual increase with some fluctuations in this process, without detecting large insect outbreaks from one week to the next.

TABLE 3. Correlation values between the estimated and the observed population values from May 2009 to November 2010.

		Nymphs_Obs	Adults_Obs
Nymphs_Est	Correlation	0.370**	
	Next (bilateral)	0.00	
Adults_Est	Correlation		0.570**
	Next (bilateral)		0.00

** Correlation is significant at the 0.01 Level (bilateral).

For the degree of similarity between the population estimates (Nymphs_Est and Adults_Est) and the field data (Nymphs_Obs and Adults_Obs) evaluated using the correlation value, Table 3 shows that the simulation of adults had a greater degree of adjustment (0.570) compared to the representations of nymphs (0.37). Although the correlations were not close to one, they were significant at 99%, which indicated a good approach and could have been related to the representation of the population fluctuation trends over time.

Second validation

When evaluating the nymph and adult fluctuation from the regression model estimates for the period December, 2010 to March, 2011 in comparison with the observations (Fig. 3 and Fig. 4), the simulation indicated increase and decrease periods correctly even though there was a difference in the number of insects. This allowed for the identification of the conditions and the moments in which the organisms began to grow; this information is useful for making decisions. For this case, a positive correlation coefficient of 0.68 was obtained for nymphs and 0.75 was obtained for adults

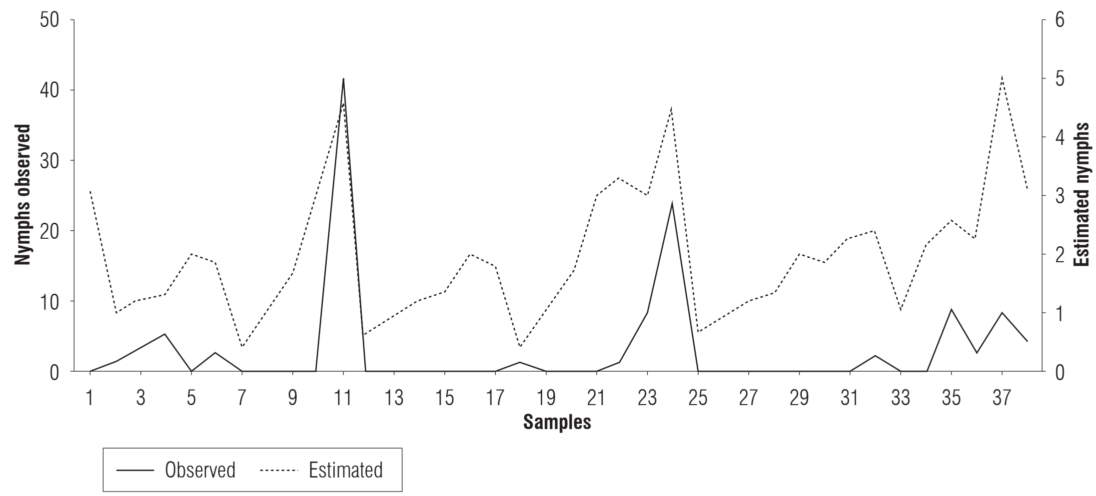

FIGURE 3. Comparison of population fluctuation observations and estimates of *Collaria scenica* nymphs from December, 2010 to March, 2011.

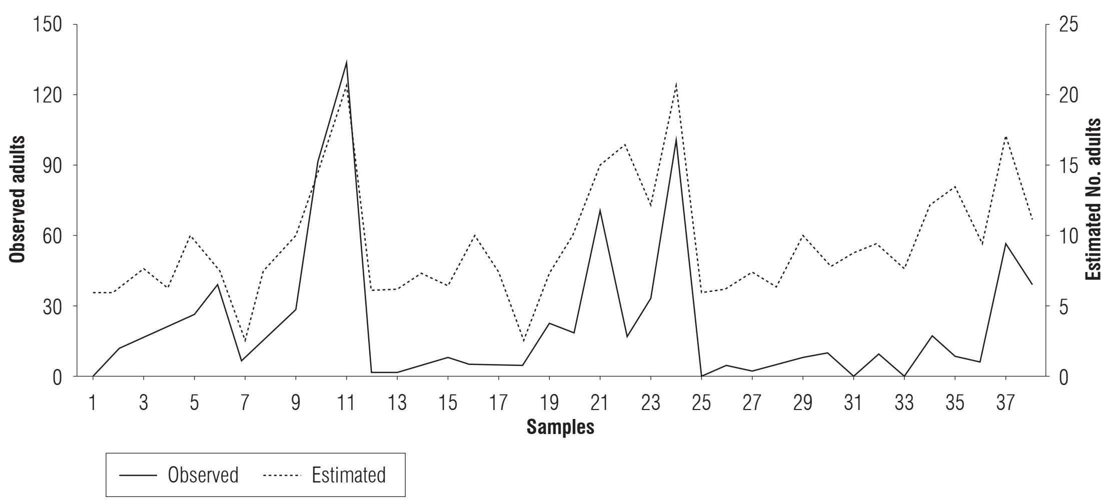

FIGURE 4. Comparison of population fluctuation observations and estimates of *Collaria scenica* adults from December, 2010 to March, 2011.

(Tab. 4). Both coefficients were significant with a 99% confidence, with the correlation true at the 0.01 level.

TABLE 4. Correlation values between the estimated and the observed population values from December 2010 to March 2011.

		Nymphs_Est	Adults_Est
Nymphs_Obs	Correlation	0.680**	
	Next (bilateral)	0.000	
Adults_Obs	Correlation		0.756**
	Next (bilateral)		0.000

** Correlation is significant at the 0.01 Level (bilateral).

Relationship between the insect population and the agroclimatic variables defined in the model

Figures 5-10 show the daily estimation of nymphs and adults for the twelve months of the year separated into agroclimatic quarters. The highest number of insects was observed in the first rainy season of the year (MAM), which is related to high temperatures and a larger daily thermal amplitude (understood as the difference between T_{max} and T_{min}) during the DJF quarter (Fig. 7), when there is accumulation of degree days that favors pest growth. Pests will then benefit from the abundance of food (forage) that occurs in the following quarter (MAM) as a result

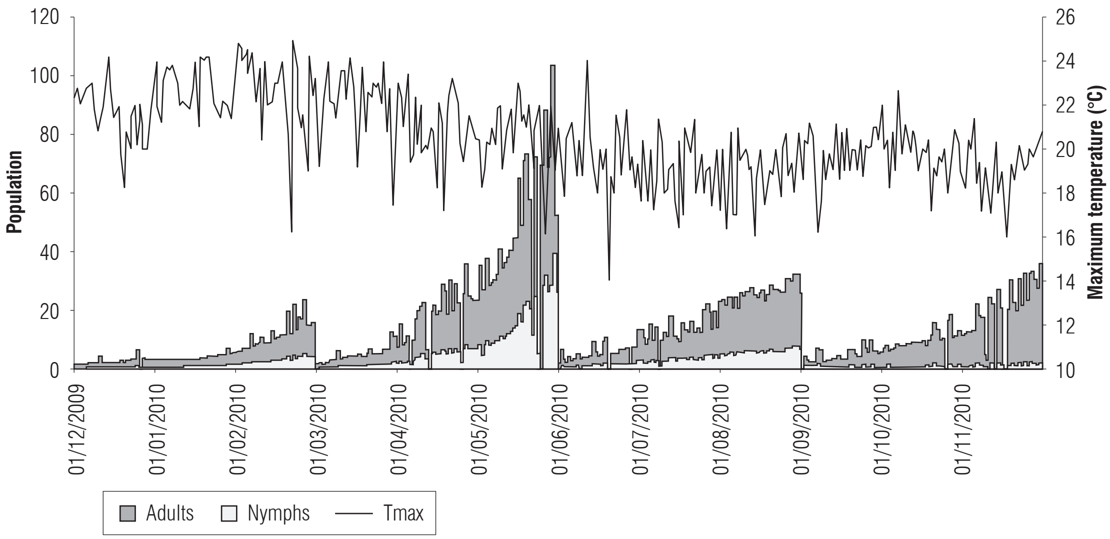

FIGURE 5. Relationship between the estimated population values of nymphs and adults and the maximum temperature (°C) from December, 2009 to November, 2010.

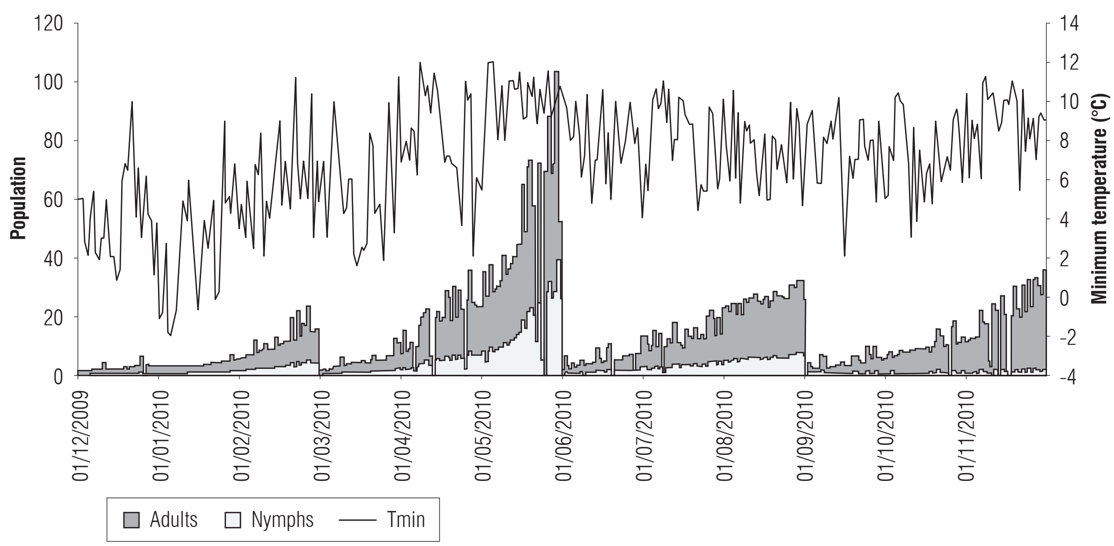

FIGURE 6. Relationship between the estimated population values of nymphs and adults and the minimum temperature (°C) from December, 2009 to November, 2010.

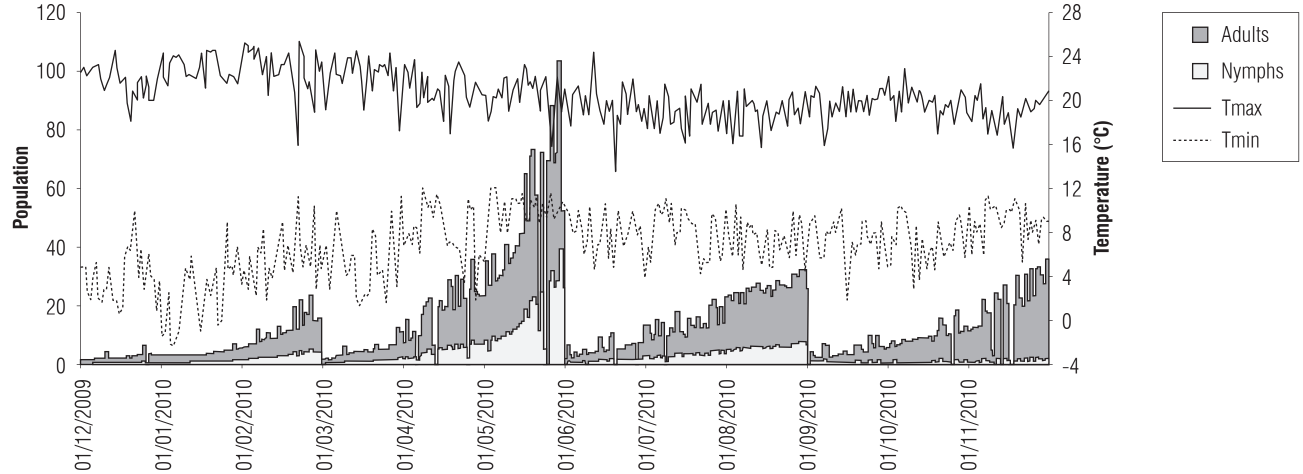

FIGURE 7. Relationship between the estimated population values of nymphs and adults and the maximum and minimum temperature difference (°C) from December, 2009 to November, 2010.

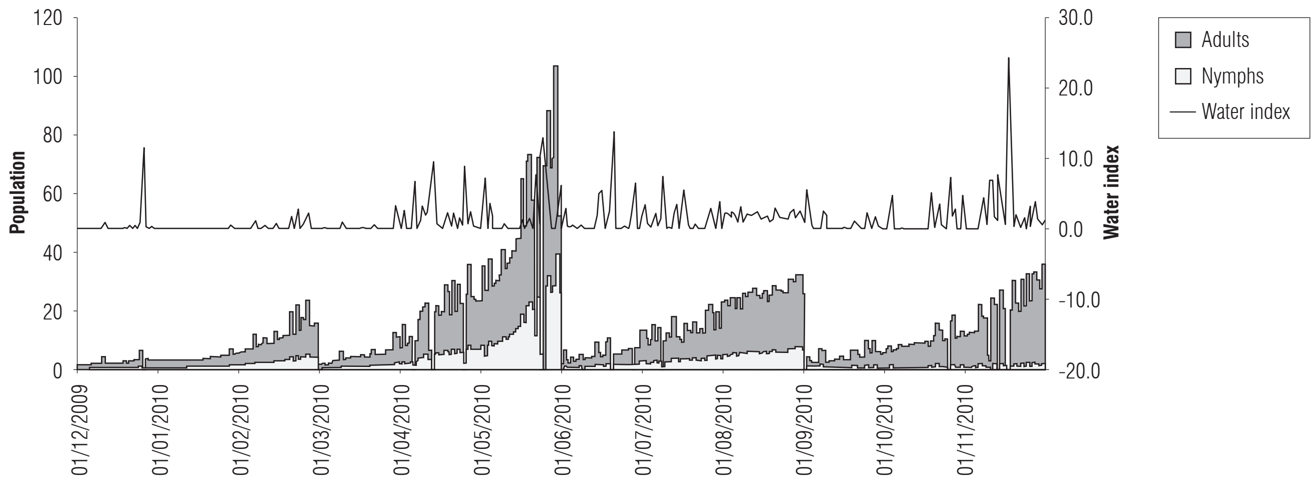

FIGURE 8. Relationship between the estimated population values of nymphs and adults and the water index from December, 2009 to November, 2010.

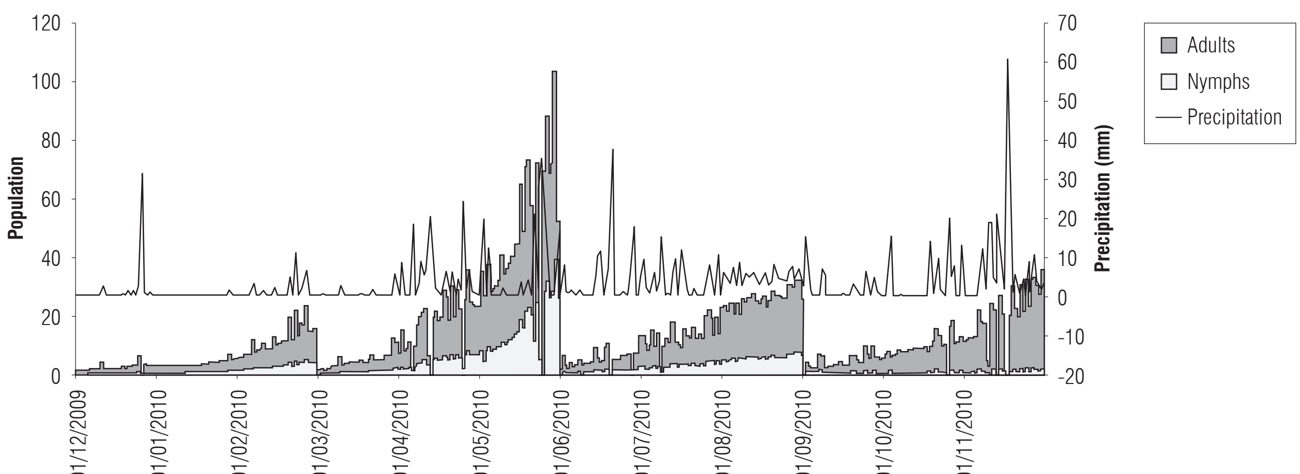

FIGURE 9. Relationship between the estimated population values of nymphs and adults and precipitation (mm) from December, 2009 to November, 2010.

of higher rainfall, which coincides with the population fluctuation of the rice bug *Oebalus insularis* (Stal), which has higher numbers during the rainy season (Vivas *et al*., 2010). These results agree with what has been indicated by cattle ranchers of the region, which relate the end of the dry season and the beginning of the rains with an increase in insect populations. Producers point out that high humidity zones and flooded areas found in certain lots are preferred by this pest.

In the analysis of the relationship between the population variation and water availability, *IH* was compared with pest growth over time (Fig. 8). The results showed that a positive effect was established in the development of this insect as a result of a larger population for the MAM quarter, in which there was higher water availability.

If population fluctuations are directly related to precipitation (Fig. 9) and evapotranspiration (Fig. 10), the former did not present a clear relationship, although for some rainfall increases, the pest population tended to grow, especially for adults. In the case of *ETo*, there was an inverse response, as during the MAM quarter, with a larger population, the *ETo* value is lower. On the other hand, for the DJF quarter with high evapotranspiration, the lowest number of nymphs and adults was found, as explained by the water availability that is used by this species in pastures.

However, it is important to note that, for insect development, although there was water availability resulting from significant rain, a previous phase of temperature accumulation was necessary since this pest grows as it stores degree days.

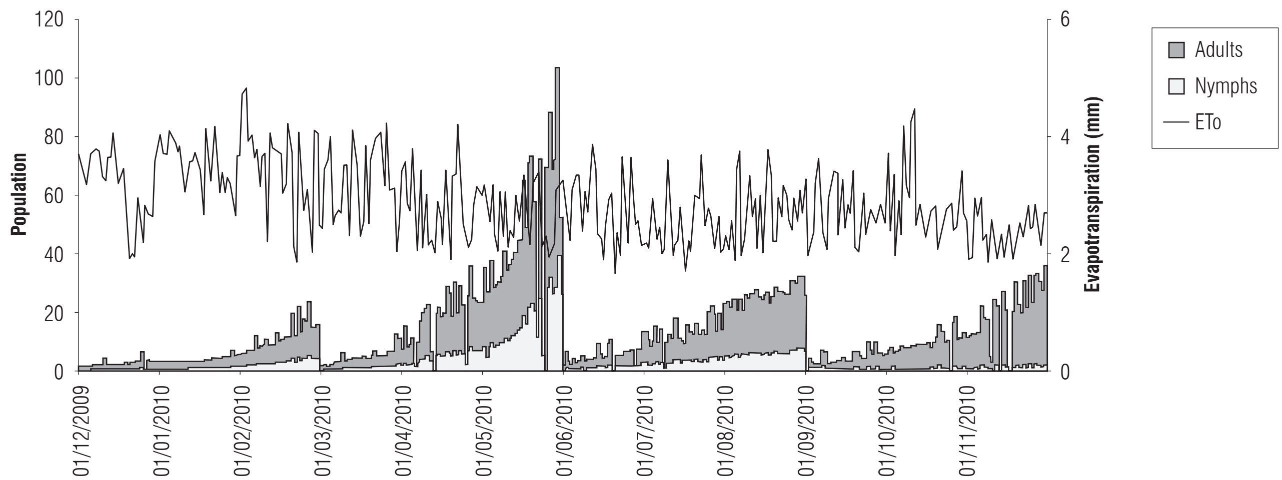

FIGURE 10. Relationship between the estimated population values of nymphs and adults and the evapotranspiration (mm) from December, 2009 to November, 2010.

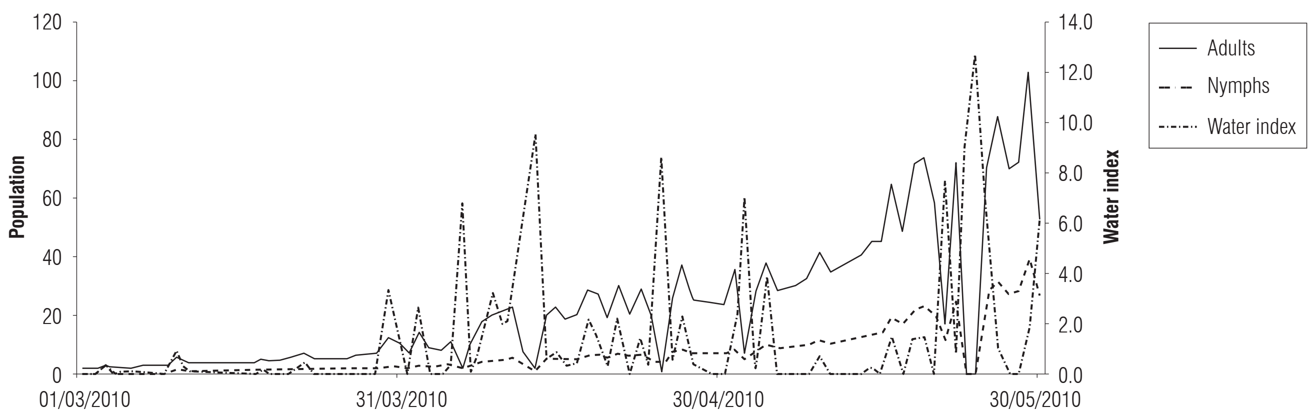

FIGURE 11. Relationship between the estimated population values of nymphs and adults and the water index from March to May, 2010.

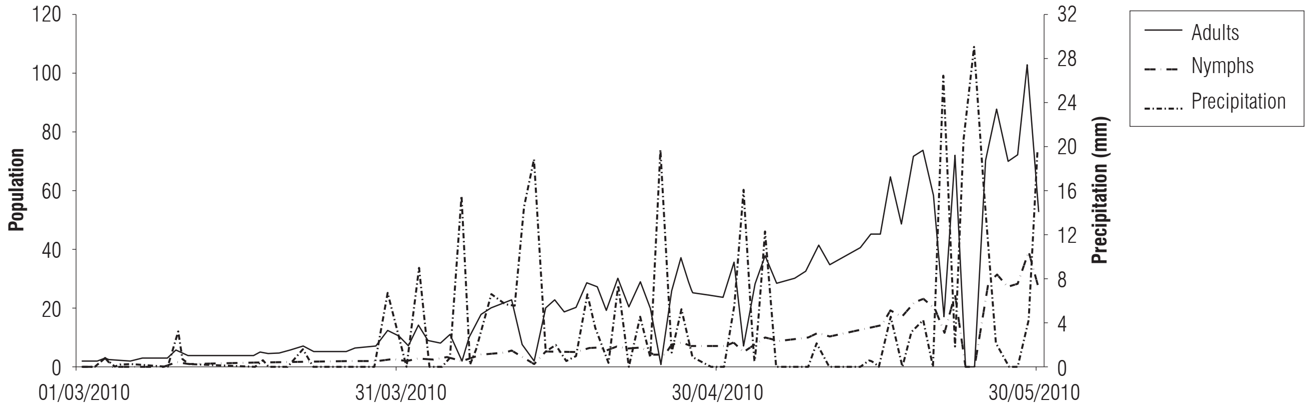

FIGURE 12. Relationship between the estimated population values of nymphs and adults and precipitation values (mm) from March to May, 2010.

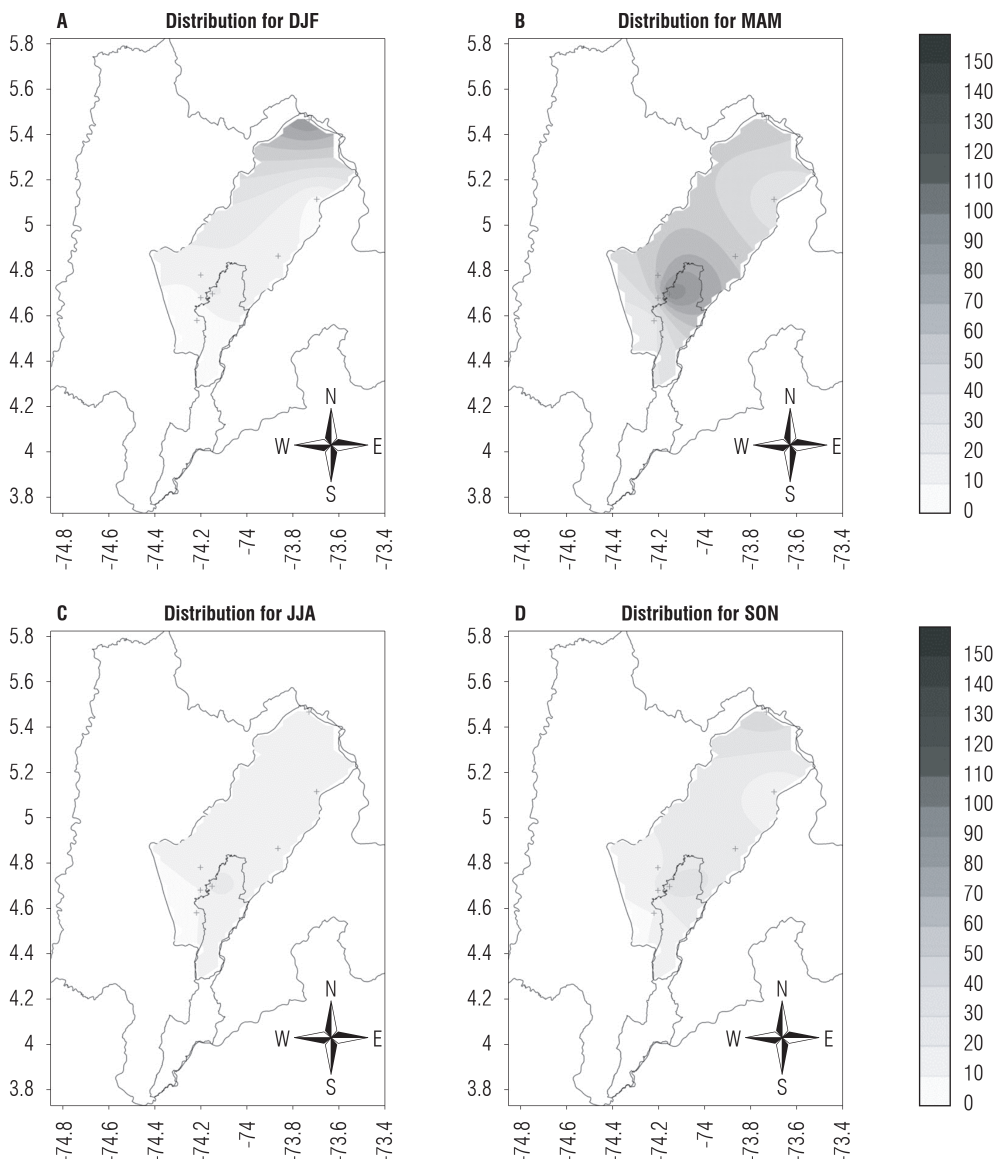

FIGURE 13. Spatial distribution of *Collaria scenica* on the Bogota plateau from the estimates for the four agroclimatic quarters considered under El Niño conditions. Abreviatures for quaters: DJF: December, January, February; MAM: March, April, May; JJA: June, July, August; SON: September, October, December. The axes represent latitude (Y) and longitude (X).

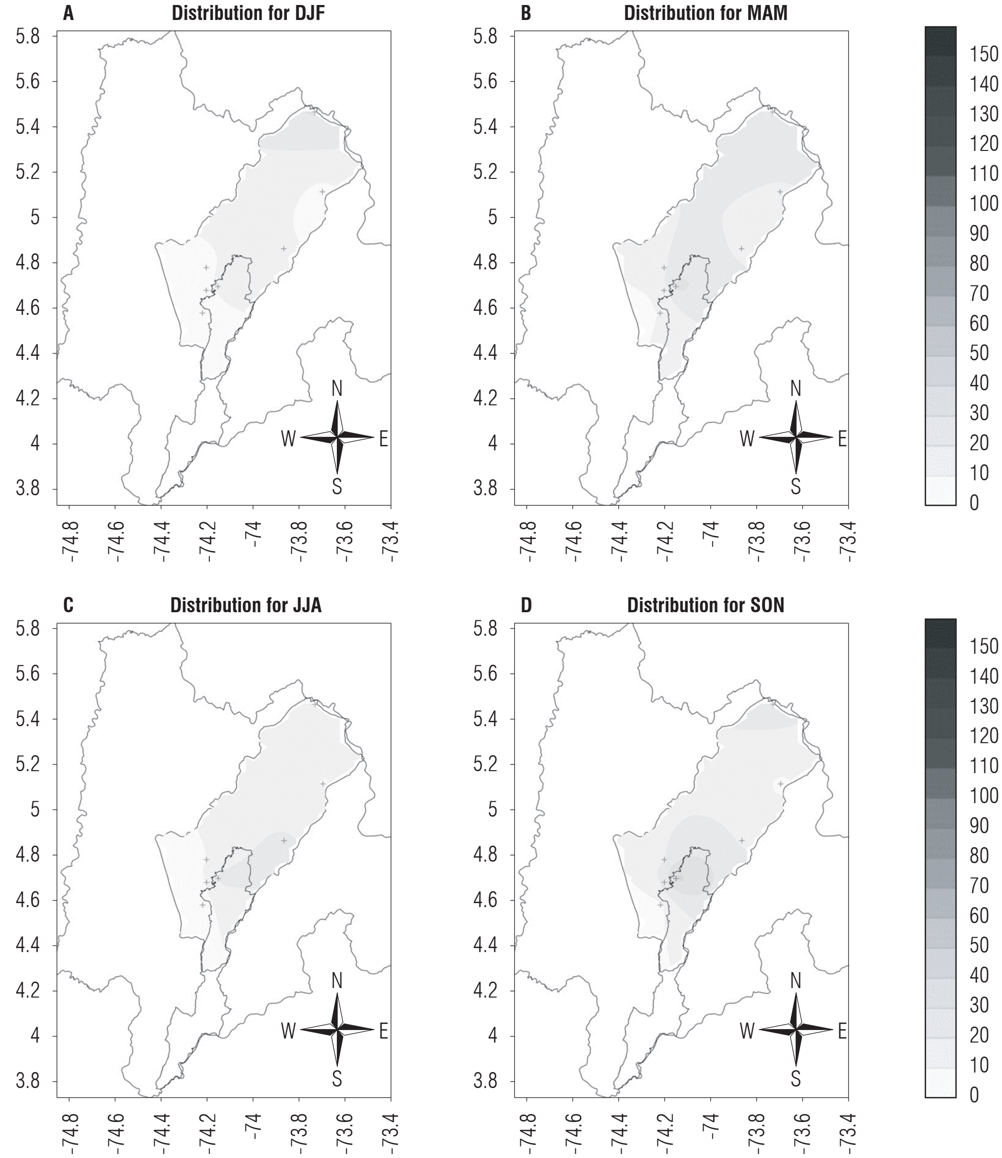

FIGURE 14. Spatial distribution of *Collaria scenica* on the Bogota plateau from the estimates for the four agroclimatic quarters considered under La Niña conditions. Abreviatures for quaters: DJF: December, January, February; MAM: March, April, May; JJA: June, July, August; SON: September, October, December. The axes represent latitude (Y) and longitude (X).

An additional analysis was performed for nymph and adult growth for the March-May 2010 period (during which the largest population of the period was found) in relation to water availability. In Figure 11, the population of MAM is shown as a function of the water index, and Figure 12 shows it in relation to precipitation. For the precipitation and index value increases, the nymph and adult population growth was recorded, but there were significant decreases in the highest peaks. Therefore, rainfall favored insects to a certain extent, and then damage ensues. According to Martínez and Barreto (1998), rain affects nymphal stages as a result of the blow of the water drop on the insect, which causes death. Additionally, excessive or scarce water is related to high mortality rates. This species is susceptible to the amount of water or its accumulation, which can also be linked to a response to the increase in food availability. When pastures have a certain amount of water, they become richer in nutrients and, therefore, become a more beneficial environment for insect species that increase their populations.

Population estimation and spatial distribution for an 'El Niño' event

To provide information on the spatial behavior of the species and establish the areas of the Bogota plateau in which climatic conditions are more favorable for this pest,

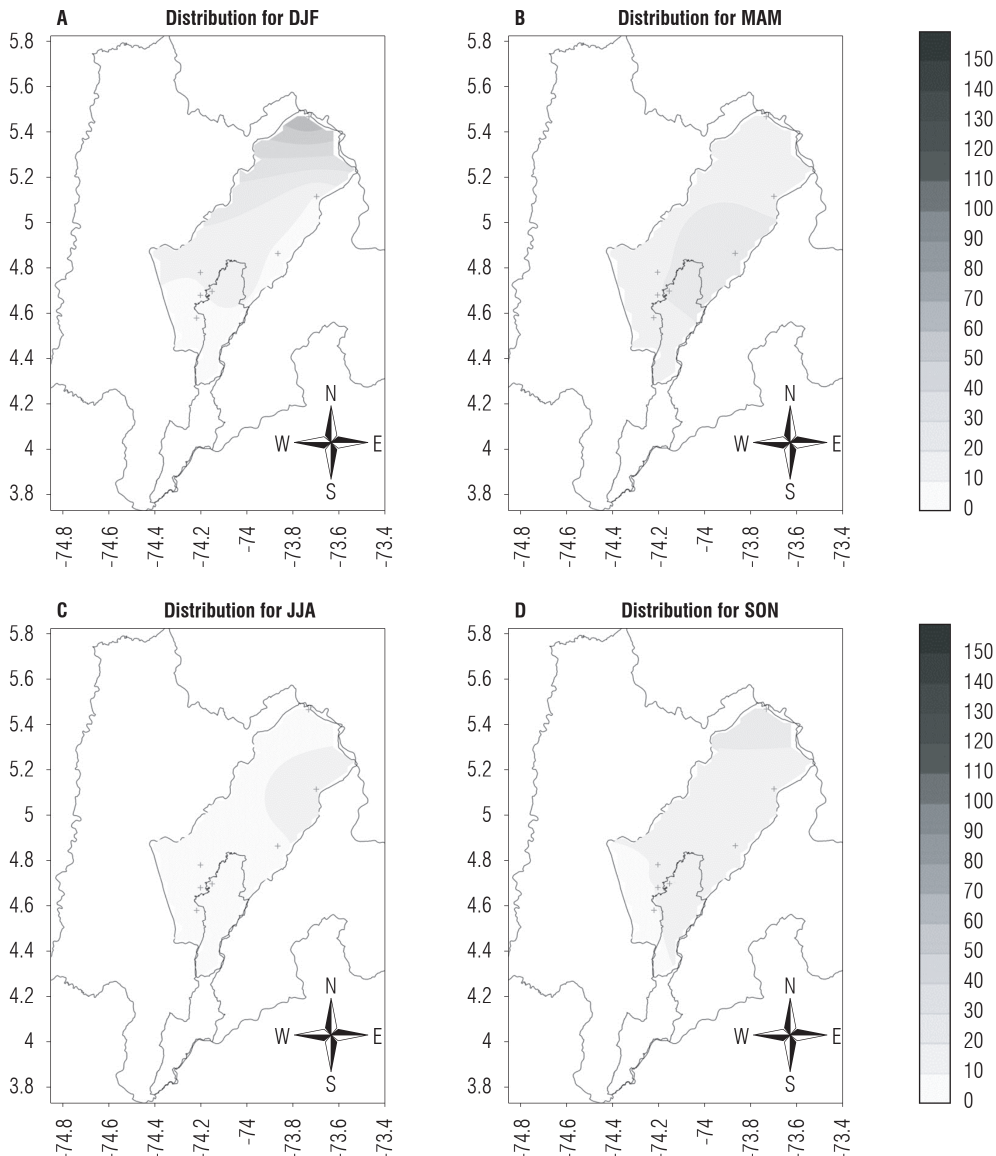

FIGURE 15. Distribution of *Collaria scenica* on the Bogota plateau from the estimates for the four agroclimatic quarters under neutral year conditions. Abreviatures for quaters: DJF: December, January, February; MAM: March, April, May; JJA: June, July, August; SON: September, October, December. The axes represent latitude (Y) and longitude (X).

population distribution maps of the region were prepared for the four agroclimatic quarters under an El Niño event (Fig. 13). In this map, the total population of this pest was considered, i.e. the sum of nymphs and adults, constructed from the data obtained from the meteorological stations. The maps included a scale from zero (0) to 150, where zero indicates the smallest population size and 150 the largest, which indicated that, under an El Niño event, the period with the highest pest presence was the MAM quarter, and the highest values were found in the southern area of the Bogota plateau.

Population estimation and territorial distribution for a 'La Niña' event

Under the La Niña conditions (Fig. 14), this pest maintained a higher presence during the rainy season, but with a lower tendency to grow than in the case of an El Niño event. This is related to water abundance, but, since there is low energy availability during a La Niña event, there is not enough heat accumulation for better pest growth. On the other hand, during an El Niño event, the amount of energy is higher because of an increase in atmospheric temperature, and water availability is also higher during the rainy seasons, favoring the development of this species.

Population estimation and territorial distribution for a neutral year

The spatial distribution on the Bogota plateau in each quarter under neutral conditions during one year (Fig. 15) presented a very similar behavior throughout the year in terms of pest presence. These values were lower than the ones found during a La Niña period and even much lower than during an El Niño event. However, in the first quarter, there was a higher pest abundance.

These results coincide with similar studies on other species in Colombia, such as the case of the coffee berry borer (*Hypothenemus hampei*). According to Ramírez *et al.* (2015) the El Niño and La Niña phenomena have a marked effect on the thermal time accumulation of this species. Above 1,400 m a.s.l., El Niño generates a significant tendency to increase favorable conditions for the growth and development of this borer.

Conclusions

An adequate method to explain the behavior of the grass bug in relation to agroclimatic variables is using a multiple linear regression equation, which considers total population values and the degree day and water indices, which builds a model with appropriate estimates of nymph and adult population fluctuations, which is useful in the prediction and analysis of the growth trends of this pest in response to the climatic conditions of the region. The degree day index establishes the optimum conditions for this species, and the water index facilitates the evaluation of possible periods with water deficiency and excess that will affect, in one way or another, the development of both the insect and the pasture.

The climatic condition characteristics in which this pest has a tendency to increase and from which population monitoring can be carried out are based on the fact that T_{max} acquires an inverse effect on the development of the pest, whereas water availability and T_{min} have a positive effect. Population growth occurs for low T_{max} and ETo values and high T_{min} and PPT values, differentiating the positive effect of abundant precipitation, but not excessive rainfall, which decreases the number of insects, possibly as a result of the blow of the drops.

The rainy seasons of the year corresponding to the MAM and SON quarters for the Bogota plateau are the highest vulnerability periods as a result of the presence of the pest. Furthermore, the first quarter had the highest abundance, which was related to an increase in temperatures during the DJF quarter, which favors pest growth and food abundance, as a result of higher precipitation values during the MAM quarter.

Under El Niño and La Niña events, population increases are estimated with higher abundance for the former because of heat accumulation resulting from increases in temperature, which favor the growth of this insect during this event. The quarter with the highest tendency to obtain appropriate climatic conditions for this grass bug was the first rainy season, which was related to a better pasture development as a result of the presence of rain and the use of temperature accumulated under El Niño conditions. During a neutral year, pest estimation was lower, and, therefore, the influence of the effects of interannual climate variability on the behavior of this grass bug on the Bogota plateau can be deduced.

Acknowledgments

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Prioritization on cultivation and climate change adaptation techniques: a potential option in strengthening climate resilience in South Africa

Priorización de técnicas de cultivo y adaptación al cambio climático: una opción potencial para fortalecer la resiliencia climática en Sudáfrica

Oduniyi Oluwaseun Samuel[1*], Antwi Micheal Akwasi[1], and Tekana Sibongile Sylvia[1]

ABSTRACT

Numerous challenges currently experienced in the world today stemmed from global scientific collaborations that rely mainly on the ecosystem. Impact of climate variability threatens food security and production especially among the rural farming households. The study was conducted in North West Province of South Africa, to identify climate change adaptation techniques and to analyze prioritization of farmers on cultivation, both in the past and present. A total number of 497 rural household maize farmers were selected through a stratified sampling method from two district municipalities. Descriptive statistics were used to compute the mean, frequency and percentages, while Wilcoxon sign rank test established farmers' prioritization on cultivation. The results show different adaptation strategies used. On the other hand, Wilcoxon sign rank test showed a statistically significant difference (P<0.05) between the farmers prioritization on cultivation both in the past and present. The study recommends incorporation of conservation agricultural practices to the existing strategies.

Key words: climate variability, maize production, Wilcoxon test, North West Province.

RESUMEN

Los numerosos desafíos que se experimentan actualmente en el mundo provienen de colaboraciones científicas globales que se basan principalmente en el ecosistema. El impacto de la variabilidad climática amenaza la seguridad alimentaria y la producción, especialmente entre los hogares de agricultores rurales. El estudio se realizó en la Provincia Noroeste de Sudáfrica, para identificar técnicas de adaptación al cambio climático y analizar la priorización de los agricultores en el cultivo, tanto en el pasado como en el presente. Un total de 497 productores de maíz de hogares rurales fueron seleccionados a través de un método de muestreo estratificado de dos municipios del distrito. Las estadísticas descriptivas se utilizaron para calcular la media, la frecuencia y los porcentajes, mientras que la prueba de clasificación de Wilcoxon estableció la prioridad de los agricultores en el cultivo. Los resultados muestran diferentes estrategias de adaptación utilizadas. Por otro lado, la prueba de clasificación de signos de Wilcoxon mostró una diferencia estadísticamente significativa (P<0.05) entre la priorización de los agricultores en el cultivo, tanto en el pasado como en el presente. El estudio recomienda la incorporación de prácticas agrícolas de conservación a las estrategias existentes.

Palabras clave: variabilidad climática, producción de maíz, prueba de Wilcoxon, provincia Noroeste.

Introduction

Global challenges currently experienced in the world today stemmed from global scientific collaborations that rely mainly on the ecosystem. The upshots gave rise to the excessive and formidable environmental problem cited by Udenyi (2010). Climate variability describes the way in which climatic elements such as temperature and rainfall fluctuate over a period. It is a variation around the mean or average that can occur in regular cycles over the years, or more randomly without any specific patterns. In sub-Saharan Africa (SSA), climate variability is the principal cause of changes in food production, South Africa inclusively (IPCC, 2007). In South Africa, climate variability has been responsible for periods of surplus in grain production as well as periods of poor production during which grains have had to be imported. Climate change can affect climate variability by increasing the frequency of extreme climatic events such as extreme temperature, drought, and flood. Climate change is a threat that further exacerbates the already precarious living conditions of many smallholder farmers (Donatti *et al.,* 2018).

Agriculture is highly exposed to climate change, as farming activities directly depend on climatic conditions. It follows that global climate change impact on agricultural

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production should be considered important (Rosenzweig and Parry, 1994). Several studies reviewed that climate change and variability pose a negative threat to agricultural production and food security. According to Bilham (2011), it was reported that temperature had more impact on the yield of the crop. The impact of climate change is very likely to affect food production at the global, regional, and local level. In every society, agriculture and food are issues that are very sensitive to climate change variability. Naturally, climate change will have overarching impacts on crop, livestock and fisheries production, and will increase the prevalence of crop pests (Campbell *et al.,* 2016). International Fund for Agricultural Development (IFAD, 2009) reported that, in Asia, recurrent and extreme events will be experienced such as droughts and floods, which are anticipated to make maize production even more problematic. It was predicted that a change in climate will put about 49 million people at risk of hunger by 2020. In the same train of thought, maize production in Africa and Latin America due to the impact of climate variability would be reduced by 10% by the year 2055 (Jones and Thornton, 2003).

The Intergovernmental Panel on Climate Change (IPCC, 2007) revealed a comprehensive appraisal of the likely outcomes of climate change on agriculture in the African region. The report depicted that Africa will be the most susceptible to climate change due to numerous stresses such as poor infrastructure, poverty, governance, amongst others. FAO (2009) stated that climate change is unfolding as a central challenge to the advancement of agriculture in Africa. The impact of climate change on maize production is becoming more elongated in the drylands of Southern Africa. The occurrence of drought is anticipated to escalate on account of higher temperatures and reduced rainfall. IPCC (2001) confirmed there is a prevalent tendency for an increase in temperature in different parts of the subregion, in association with climate variability and extreme weather events.

This impact of climate change affects maize production in South Africa. According to Grain South Africa (GSA, 2010), the industry is one of the largest food supplies, producing between 25 and 33% of the country total gross agricultural production. However, the current situation as a result of climate change has led to a drastic decrease in the production of maize. The climate situation, which is becoming hotter and drier, will generate a remarkable decrease in the production of maize by approximately 10-20% over the next 50 years (BFAP, 2007). Following the current trends of rainfall patterns, maize production would be adversely affected by the impact of climate change. The current inconsistency of patterns in weather in South Africa could consequently have a substantial negative impact on the maize economy (Mqadi, 2005). Over the last few years, there has been a major shift in area and production of maize in South Africa. The areas where maize is planted have declined significantly.

In the view of this, adaptation practices are considered as a technique worthy of strengthening climate resilience among rural household maize farmers in the study area. Climate impacts and adaptation strategies are the major distress area to the body of science; as such, it is paramount that farmers should possess the ability to perceive the incongruity associated with climate, for it is a requisite for the adoption of adaptation (Moyo *et al*., 2012; Kihupi *et al*., 2015). As postulated by Adger *et al.* (2005), in a bid to combat climate change through the implementation of adaptation, necessity is laid upon the farmers to first perceive a change in climatic condition after which there is a need to identify and apply potential useful adaptations. According to Kihupi *et al.* (2015), adaptation strategies of smallholder farmers largely depend on their level of perception knowledge on climate change. However, several studies have been conducted around the globe on how smallholder farmers adapt to climate variations and the significance of adapting agriculture to climate change in the continent (Deressa *et al.,* 2009; Mertz *et al.,* 2009; Hisali *et al.,* 2011; Kemausuor *et al.,* 2011; Below *et al.,* 2012).

There are various adaptation practices to implement in the face of climate change impacts. In this regard, Osbahr *et al.* (2010) opined that crop varieties and livelihood diversification are some of the major adaptation measures adopted by farmers throughout the continent. In India, there are some noticeable changes in the agricultural practices (maize farming), which include adaptation strategies such as groundwater for irrigation and the use of PVC pipes to transport water on farms (Mudrakartha, 2012). Other methods are the use of early matured cultivars, the increase in the use of high yield crop varieties, change in planting date and harvesting, crop diversification, mix-cropping, and agroforestry. Improving irrigation facilities and introducing cultivars were identified by Wang *et al.* (2001) in a research conducted on maize farming adaptation measures in China. However, adaptation options are subjective to different environmental factors such as flood, drought, extreme weather condition, etc. (Gbetibouo *et al.,* 2010; Hisali *et al.,* 2011; Below *et al.,* 2012).

According to Deressa *et al.* (2011), adaptation measures used in the Eastern coast of Africa in maize farming were the utilization of different maize cultivars, irrigation, and change of planting dates. Equally, Mary and Majule (2009) reported that, in Tanzania, the rural farmers adapt by simply changing the date of planting. Furthermore, the rural household in Tanzania engages in the burying of crop residues to improve soil fertility as well burning the residues to control pest infestation. Additionally, in SSA diversification of livelihood strategies to non-farm activities were practiced. In southern Africa, Zvigadza *et al.* (2010) reported that, in Zimbabwe, traditional coping strategies were identified with the aim of adapting to the aftereffects of climate change. The use of water recycling on the farm, the indigenous method of water conservation, practicing spiritual exercise requesting for rain were all used. According to Ndhleve *et al.* (2017), in South Africa, supplementary irrigation and change of planting date were identified for adaptation strategy. Farmers engage in the adaptation by re-planning or shifting the planting date to earlier or later moments; additionally, the use of forecasting and weather reports were all measures used.

The impact of climate change on farmers' production brought about prioritization of farmers determinant to cultivation. Today, farmers' priority on cultivation has changed due to climatic events. Farmers considered some activities imperative in the present world of farming unlike in the past. This study seeks to provide an insight to farmers' prioritization to cultivation both in the past and present, as no study of such has been carried out in the area. The objectives of this study were to identify various adaptation techniques and analyze farmers' prioritization on cultivation in the past and present, used among the rural household maize farmers in the study area.

Materials and methods

Study area

The study was carried out in the North West Province, which lies in the north of South Africa on the Botswana border, with the Kalahari Desert to the west, Gauteng province to the east and the Free State to the south. Its landscape is demarcated by Magaliesberg Mountain in the northeast, which extends to about 130 km from Pretoria to Rustenburg, while the Vaal River forms the province's southern border. The region is flat and consists of grassland and bushveld scattered with trees and shrubs, with the capital city situated in Mahikeng and the largest city is Rustenburg. A summer-rainfall region, in which temperatures range from up to 31°C in summer to 3°C in winter. Mahikeng (previously Mafeking) is the capital and most economic activity in the province is concentrated in the Southern region between Potchefstroom and Klerksdorp, as well as Rustenburg and the Eastern region, where more than 80% of the province's economic activity takes place.

North West Province comprises four district municipal councils which are in turn divided into 18 local municipalities. The largest is Bojanala Platinum District Municipality covering about 18,333 km^2; the other include Ngaka Modiri Molema District Municipality, Dr. Ruth Segomotsi Mompati District Municipality, and Dr. Kenneth Kaunda District Municipality. However, the study was carried out in two district municipalities which are Bojanala and Ngaka Modiri Molema District Municipality. Bojanala district comprises five local municipalities, which include: Rustenburg, Moretele, Kgetleng, Moses Kotane, and Madibeng. The population of the district is approximately 1.5 million. On the other hand, Ngaka Modiri Molema District consists of Mahikeng, Ditsobotla, Ramotshere Moiloa, Tswaing, and Ratlou. The area of the district is 28,206 km^2 with a population 842,699. Farming is most predominant in these areas and they are known as the first largest white maize producing areas in the country. Other farming activities include planting of sunflowers, rearing of cattle and vegetables.

Figure 1 shows the two districts where the study was carried out including their local municipalities.

Method of data collection

Permission to collect data was granted by the districts and local municipalities together from the rural household heads and the extension officers to conduct the research. The data used in the research was primary data which was collected in the year 2016. Data was collected through face-to-face interviews with the farmers, in which 497 questionnaires were administered in the research area. A well-structured questionnaire written in English was used as a research tool to collect data. This tool was selected because of its low cost and the little expertise required to run. The questionnaires were tested and validated before the final administration to the respondents. The questionnaires were explained to the local extension officers before the survey because they understand the farmers better and can translate the questionnaires into a local language. Face-to-face interviews and focus group discussion was conducted in each local municipality where each session lasted for 45 minutes. The questionnaires were filled in anonymously as no personal questions regarding names, addresses and identity numbers were asked. Questionnaires consisted of a logical flow of questions which addressed matters related to (a) demographic characteristics, (b) land characteristics,

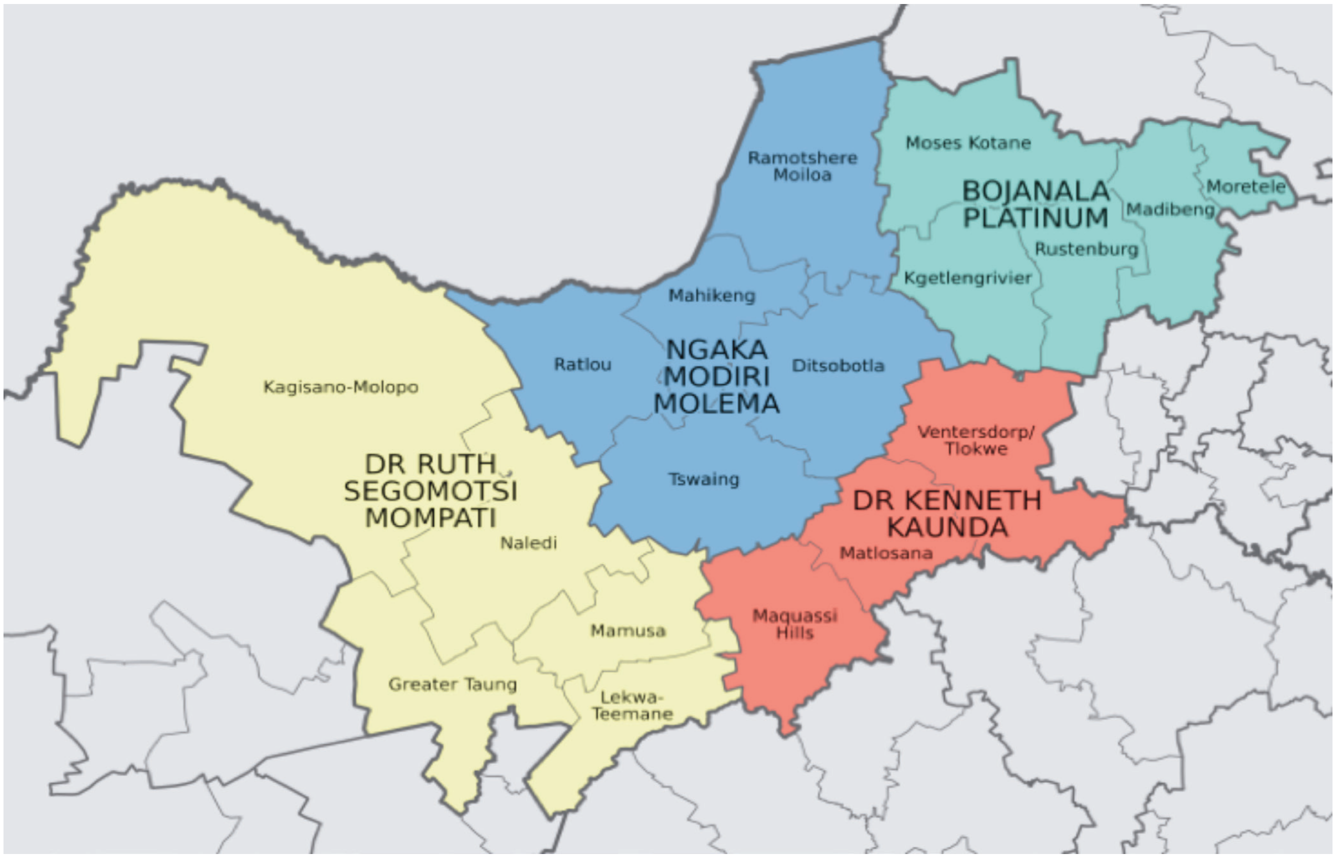

FIGURE 1. Districts and local municipalities in North West Province. Source: Municipality and Demarcation Board of South Africa (2009).

(c) climate change related issues, such as climate change perception and adaptation practices, and (d) farmers' prioritization of determinants of cultivation in the past and present. Furthermore, the distribution of rainfall patterns and temperature across the study area were accessed and collected from South African Weather Services.

Population, sampling procedure, and sample size

The research was designed in such a way that data were collected from two district municipalities (Bojanala District and Ngaka Modiri Molema) in North West Province, which consists of 10 local municipalities altogether. The list of small-scale maize farmers in the two districts was obtained from the Department of Agriculture, Forestry, and Fisheries (DAFF). Raosoft sample size calculator was used to determine the sample size from the population of the small and emerging maize farmers in the study area. *Raosoft* presents a *sample size calculator* that takes into account the margin of error, the confidence level, and the response distribution.

The calculation of the sample size *n* and margin of error *E* are given by:

$$x = Z\,(^{c}/_{100})^{2}\, r\,(100-r) \quad (1)$$

$$n = {}^{Nx}/_{((N-1)E^{2}+x)} \quad (2)$$

$$E = \mathrm{Sqrt}\,[^{(N-n)\,x}/_{n(N-1)}] \quad (3)$$

Stratified random sampling technique was used to group the population of the farmers from the 10 local municipalities in the two districts into strata, after which a random sample was used to select from each stratum. A specific number of sample sizes was selected from the population from each local municipality. A total of 497 questionnaires were administered in the two districts to participate in the research study.

Statistical analysis

Data collected were analysed using the Statistical Package for Social Sciences (SPSS, version 23 of 2015) software. SPSS software can be used to assist in calculating a variety of statistical analysis which has a dynamic data processing ability. The data were subjected to descriptive statistics such as frequency, percentages, mean and graphical representations. These were employed to analyze the household demographics information and observe climate variability and adaptation strategies in other to fulfill the objectives of the study. On the other hand, Wilcoxon Sign-rank Sum Test was used to analyze farmers' prioritization of determinants on cultivation in the past and present.

Farmers' prioritization of determinants on cultivation using Wilcoxon Signed-rank computation

The Wilcoxon Signed-rank Sum Test applies to two-sample designs involving repeated measures, matched pairs, or "before" and "after" measures like the t-test for correlated samples. The Wilcoxon Signed-rank Test is a non-parametric version of a paired samples t-test, used to test the difference between paired data and it compares two groups. The Wilcoxon Signed-rank statistics can be computed as sign statistic of the pair-wise averages of data (Hettmaspherger *et al.,* 1997).

Null hypothesis: There is no statistically significant difference between the two variables. Farmers' prioritization on cultivation in the past is same with cultivation in the present.

Alternative hypothesis: There is a statistically significant difference between the two variables. Farmers' prioritization on cultivation in the past is not the same with cultivation in the present.

Empirical Model: $M = \Sigma X / N$

Formula for the normal distribution:

$$\frac{f(x) = e^{-(x-\mu)^2/2\sigma^2}}{\sigma\sqrt{2\pi}} \quad (4)$$

For a given mean (μ) and standard deviation (σ), plug in any value of x to receive the proportional frequency of that value in that particular normal distribution.

With sample taken from Population A being smaller than the sample from Population B) - reject H_0 if $T_A \geq T_U$ or $T_A \leq T_L$

Results and Discussion

Distribution of municipalities

Table 1 below shows the distribution of households according to the municipalities. Most of the farmers interviewed were from Ngaka Modiri Molema with 76.5% of the total sample. This area is known for maize production in the country with five local municipalities namely; Tswaing, Ditsobotla, Mahikeng, Ratlou, and Zeerust. Most maize farmers were from Tswaing local municipalities with 25.2%, followed by Ditsobotla with 21.1%, Mahikeng with 15.7%, Ratlou with 2.0% and Ramotshere Moiloa, which is the smallest with 1.8%. On the other hand, Bojanala District constitutes 23.5% of the total respondents, with 5 local municipalities which include; Kgetleng with 8.9%, Rustenburg is 9.1%, Moses Kotane constitutes 7.0%, Madibeng is 6.0% and Moretele with a proportion of 3.2% as shown below.

TABLE 1. Distribution of the municipalities.

Characteristics	Frequency	Percentage
District municipalities		
Bojanala District	117	23.5
Ngaka Modiri Molema	380	76.5
Total	497	100.0
Local municipalities		
Kgetleng	44	8.9
Rustenburg	45	9.1
Moses Kotane	35	7.0
Madibeng	30	6.0
Moretele	16	3.2
Tswaing	125	25.2
Ditsobotla	105	21.1
Mahikeng	78	15.7
Ratlou	10	2.0
Ramotshere Moiloa	9	1.8
Total	497	100.0

Demography

The findings regarding demographics in the study area are shown in Table 2. These include information about farmer's household size, gender, age, marital status, educational background and the source of income. Results of the survey in Table 2 showed that 76.3% of the farmers were male, while 23.7% were female. As regards to marital status, 26.6% were single, 54.7% were married, 8.0% were divorced, and 5.8% were widows, while 4.8% was separated. Agriculture provides food and fiber for the people; 67.4% of the farmers have their major source of income from agriculture while 32.6% do not have their major source of income from agriculture. The results for the age group indicated that the majority of the farmers fall within the age group of 61-70 years old. According to Bayard *et al.* (2007), age is positively related to some climate change adaptation measures that are related to agricultural activities. The age group 41-50 constitutes 24.9%, while the smallest age group is 71-80 with 1.2%. The youth age group (18-30 years) constituted 8.5% and they seem not to be interested in farming. The computer age enables young people to divert attention from agriculture into information technology and other related professions. This result confirmed to the findings of Maponya and Mpandeli (2012), who stated that young people in the communities are involved in other activities and use opportunities in the fields of information technology, tendering and jobs in various government departments in the province.

TABLE 2. Demography (composition and household characteristics).

Characteristics	Percentage
Household size	
1-3	30.6
4-6	39.4
7-9	21.1
10-12	4.6
13-15	4.2
Total	100.0
Household gender	
Male	76.3
Female	23.7
Total	100.0
Household age	
18-30	8.5
31-40	17.5
41-50	24.9
51-60	19.9
61-70	28.0
71-80	1.2
Total	100.0
Household marital status	
Single	26.6
Married	54.7
Divorced	8.0
Widowed	5.8
Separated	4.8
Total	100.0
Educational level	
Pre-school	3.4
Sub Standard A & B	9.3
Standard 1-5	36.0
Standard 6-10	28.4
Higher	7.2
None	15.7
Total	100.0
Farming as major income	
Yes	67.4
No	32.6
Total	100.0

Education is a key to power. The result on the level of education indicated that most of the farmers fall under standard 1-5 (grade 3 to grade 7), with 36% while 15.7% has no formal education. The level of education has a significant difference in farmers' perception to climate change. According to Asfaw and Admassie (2004) and Bamire *et al.* (2002), it was reported that education affected agriculture productivity by increasing the ability of farmers to produce more output from given resources and by enhancing the capacity of farmers to obtain and analyze information. Maddison (2007) revealed that educated and experienced farmers are expected to have more knowledge and information about climate change and adaptation measures to use in response to climate challenges. About 28.4% fall under standard 6-10 (grade 8 to grade 12), while 3.4% attended preschool. Many of the household size falls under the household grouping of 4-6 household members, with 39.4%. The household size of 1-3 is 30.6%, 7-9 is 21.1%, while household sizes of 10-12 and 13-15 constitute 4.6 and 4.2%, respectively.

Adaptation measure and strategies in the study area

Table 3 shows various adaptation strategies used by the farmers in the study area. Most of the strategies above were targeted towards drought as increased temperature is the most perceived element in the study area. About 29.8% of the farmers in the study area practiced minimum or zero tillage to cope with drought by conserving soil moisture content and preserve soil organic carbon. Minimum tillage is considered to be an environmentally agricultural practice which helps to enhance the soil arrangement. Environmentally agricultural practice is defined as a practice that supports both agricultural production and biodiversity conservation, working in harmony together to improve the livelihoods of rural communities. It is one of the practices used in conservation agriculture to promote sustainability. According to Maponya and Mpandeli (2012), it was reported that farmers from Limpopo engaged in minimum tillage to cope with drought. Ndamani and Watanabe (2016) revealed by a research carried out in Ghana that a slim majority of respondents (51%) use crop diversification strategies in response to climatic variability. Changing the planting date was chosen by 22% of the respondents, while improving crop varieties was chosen by 12%. Farmers also use farm diversification measures (6%), income generating activities (6%) and irrigation (2%) to mitigate the effects of climate change on their farming activities. About 1% of the respondents also undertakes agroforestry.

From the results, it was shown that about 5.2% of the farmers practiced crop diversification. Many adaptations to climate change and variability by rural household farmers were centered on diversification. For example, Fisher *et al.* *(*2015) reported that, in a rainfed systems that are prone to drought, farm diversification could take advantage of spatial variability in rainfall. The most common diversification strategy identified by the several studies was to grow a variety of crops (Bryan *et al.,* 2009, 2013; Bele *et al.,* 2014; Westengen and Brysting, 2014). Previous research demonstrated a positive correlation between crop diversity and production (Di Falco *et al.,* 2010). The likelihood of crop diversification is positively influenced by secure land tenure, access to information and credit, labor supply, and farming experience (Hassan and Nhemachena 2008; Gbetibouo *et al.,* 2010; Fosu-Mensah *et al.,* 2012).

Another adaptation strategy from the study was the planting of different crops. About 5.2% of the respondents were involved in planting different crops to adapt to climate change, especially if one crop fails the other crops can still generate an income. Climate change will likely affect regional cropping patterns in sub-Saharan Africa (Kurukulasuriya and Mendelsohn, 2006). Many studies revealed farmers reassessed and cultivated different crops in response to perceived changes in temperature and rainfall. For example, Kenyan farmers switched to cassava, sweet potatoes, and pigeon peas (Bryan *et al.,* 2013). Cassava, in particular, is potentially useful for adaptation to climate change in sub-Saharan Africa, because it grows in marginal soils, tolerates periodic and extended periods of drought and heat, and is left in the ground until needed (Jarvis *et al.,* 2012). Malawian farmers migrated to cassava growing areas during the 2001-2002 famine (Brooks, 2014). Important factors enabling crop switching are access to irrigation and to extension information (Bryan *et al.,* 2009, 2013).

Few of the farmers are planting improved seeds. About 3.6% of the farmers engaged in plant tolerant maize seeds. Maize is the most important food crop in SSA, where it is almost completely rainfed and, therefore, dependent on the region's increasingly erratic precipitation. Around 40% of Africa's maize-growing area faces occasional drought stress in which yield losses are 10-25%. Around 25% of the maize crop suffers frequent drought, with losses of up to half the harvest. To reduce vulnerability and improve food security, the Drought Tolerant Maize for Africa (DTMA) project has released 160 drought tolerant (DT) maize varieties, between 2007 and 2013. The yield advantage of the new DT maize varieties over local maize varieties is greater. Research in SSA has indicated a consistent yield advantage of improved maize varieties over local maize varieties at different levels of fertilizer use and various soil fertility and rainfall conditions (Smale and Jayne, 2003). However, while farmers expressed a demand for drought tolerance, availability of improved DT maize and sorghum seed limited their use in several countries (Cavatassi *et al.,* 2011; Fisher and Snapp, 2014; Westengen and Brysting, 2014). Only in Nigeria farmers did have access to improved DT varieties due to the presence of two development projects (Tambo and Abdoulaye, 2013).

A change to drought-tolerant crops such as sunflowers was also practiced. Switching to crop varieties less sensitive to climatic stress is one of the preferred strategies of farmers in SSA. The study reviewed that about 2.4% of the farmers switched to drought tolerance crops. This is in accordance with Fisher *et al.* (2015), who reported that policymakers also support this approach: a UN General Assembly resolution in 2009 emphasized the development of crop varieties that tolerate environmental stresses, including drought (Westengen and Brysting, 2014). New varieties of staple crops, many still under development, provide drought and heat tolerance as well as early maturation (Karaba *et al*., 2007; Cairns *et al*., 2013). The studies revealed that improved short-season varieties were available and farmers were growing them to escape drought (Thomas *et al.,* 2007; Fosu-Mensah *et al.,* 2012; Fisher and Snapp, 2014; Westengen and Brysting, 2014).

Changing the planting dates is another adaptation strategy, with 4.2% of the farmers using this method in the study area. According to Reason *et al.,* (2005), the onset of the rainy season is crucial to the timing of rain-fed crops: if the farmer plants too early, soil moisture will be insufficient for seed germination; if the farmer plants too late, intense rain might wash the seeds away. Farmers in several SSA countries reported they shift crop planting dates in response to year-to-year variability in the rainy season onset (Sofoluwe *et al.,* 2011; Fosu-Mensah *et al.,* 2012; Bryan *et al.,* 2013; Bele *et al.,* 2014).

Crop rotation strategies were adopted by about 5.6% of the farmers. It plays an important role in increasing maize production in the study area. The result shows a similar report with other recent studies. Crop rotation or switching crops was still found to have an effect on maize productivity (Kuntashula *et al*., 2014). Crop rotations are a temporal diversity through crop rotations. For example, alternating cereal crops with broadleaf crops and changing stand densities disrupts the disease cycles (Krupinsky *et al.,* 2002). In Tanzania, farmers diversify crop types in a form of rotation as a way of spreading risks on the farm (Adger *et al.,* 2003; Orindi and Eriksen, 2005). This serves as an insurance against climate variability.

However, some strategies from Table 3 are combined by the farmers to adapt to climate change. For example, some farmers apply a combination of crop diversification, plant tolerant maize seeds, and change to drought-tolerant crops, while others apply a combination of planting mature cultivars and shorten the growing period.

TABLE 3. Adaptation strategies.

Practices	Frequency	Percentage
Minimum or low tillage	148	29.8
Crop diversification	26	5.2
Plant different crops	26	5.2
Plant tolerant maize seeds	18	3.6
Change to drought tolerance crops	12	2.4
Crop rotation	28	5.6
Changing of planting date	21	4.2
Planting in different area	3	0.6
Reduced cultivated land	7	1.4
Ripping deeper and ploughing every year	20	4.0
Prayers	23	4.6
Improved land management	7	1.4
Change of production practices	1	0.2
Combination of 2, 3 and 5	72	14.5
Combination of 12 and 13	51	10.3
None	34	6.8
Total	497	100.0

Analysis of farmers' prioritization of cultivation determinants in the past and present

Tables 4 and 5 summarize the prioritization determinants used by the farmers prior to planting in the past and at present. In the past, soil management (29%) and network with cultural groups (23.7%) were the farmers' priority. They seemed to engage in networking with a cultural association, religious groups, committees in the community, farmers' associations and other farmers. However, at present, farmers' prioritization determinant involves more infrastructural and structural facilities, acquiring new skills to cope with the impact of climate change and assessing available strategies option.

TABLE 4. Frequency table for prioritization of determinants on cultivation in the past.

Prioritization of determinants	Frequency	Percentage
Network with cultural association	118	23.7
Network with religious group	40	8.0
Network with committees in the community	61	12.3
Network with other farmers	79	15.9
Network with farmers' association	10	2.0
Soil management	144	29.0
Access to water for farm production	45	9.1
Total	497	100.0

TABLE 5. Frequency table for prioritization of determinants on cultivation in the present.

Prioritization of determinants	Frequency	Percentage
Extension service access	53	10.7
Agribusiness skill	8	1.6
Water management	74	14.9
Innovative and creative thinking	4	0.8
Decision making skill	1	0.2
Soil management	51	10.3
Access to production infrastructure	1	0.2
Network with financial institution	3	0.6
Network with farmers' association	56	11.3
Network with farmers' cooperative	40	8.0
Infrastructural facilities	65	13.1
Structural facilities	108	21.7
Education and training	33	6.6
Total	497	100.0

Tables 6, 7 and 8 show the analysis of farmers' prioritization determinants on cultivation both in the past and present. The interpretation of the tables is summarized below.

A Wilcoxon Signed-Ranks test indicated that the prioritization of determinant on cultivation present (mean rank = 267.08) was rated more favorably than the prioritization of determinant of cultivation before (mean rank = 95.87), Z = -15.434, $P = 0.000$". The Wilcoxon signed rank test shows that the observed difference between both measurements is significant. There is a statistically significant difference and

TABLE 6. Descriptive statistics.

	N	Mean	Std. Deviation	Minimum	Maximum
Prioritization of determinant on cultivation before	497	3.88	2.138	1	7
Prioritization of determinant on cultivation present	497	8.65	4.561	1	14

TABLE 7. Ranks.

	N	Mean rank	Sum of ranks
Negative ranks	96[a]	95.87	9203.50
Positive ranks	366[b]	267.08	97749.50
Ties	35[c]		
Total	497		

TABLE 8. Statistics based on Wilcoxon Signed Ranks Test.

	Prioritization of determinant on cultivation present - Prioritization of determinant on cultivation before
Z	-15.434[a]
Asymp. Sig. (2-tailed)	0.000

a. Based on negative ranks.

we reject the null hypothesis that both samples are different. We assume here that there is a difference between present and past. The mean rank for prioritization determinants at present is higher than in the past.

Conclusions

The study brought a limelight by identifying the different adaptation strategies used, which include minimum or zero tillage, crop diversification, planting different crops, planting tolerant maize seeds, changing to drought tolerant crops, crop rotation, changing planting dates, planting in different areas, reducing cultivated land, ripping deeper and ploughing every year, prayers, planting mature cultivars, shortening the growing period, improving land management and changing production practices. However, minimum tillage is the most used practice with about 29.8% of the respondents. The evidence of climate variability in the study area was established, which revealed a continuous increase in temperature and low rainfall patterns over the years. In the same manner, the study investigated farmers' prioritization of determinants of cultivation in the past and present. It showed the descriptive statistics of prioritization options used by the farmers in the past and present.

The result revealed that soil management and network with cultural groups were the most used priorities by farmers in the past. However, in the present, farmers' prioritization has been altered in other to cope with climate change scenarios and variability. At present, farmers use varieties of prioritization options ranging from agribusiness skill, water management skill, decision-making skill, innovative and creative thinking, soil management, infrastructural facilities, structural facilities, education and training, network with farmers' association, among many others. The result emphasized that there was a statistically significant difference between farmers' prioritization of determinants of cultivation in the past and present. Prioritization of determinant of cultivation at present is greater than that of the past, with a mean rank of 267.08, N is 366[b] (positive rank) and *P*<0.000.

The study recommends that present prioritization should be look to enhance adaptation, along with the incorporation of conservation agricultural practices to the existing strategies.

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The importance of non-monetary cost in start-up and annual cacao (*Theobroma cacao* L.) production activities in Santander, Colombia

La importancia del costo no monetario en el establecimiento y las actividades anuales de producción de cacao (*Theobroma cacao* L.) en Santander, Colombia

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ABSTRACT

Based on a double-phase methodology (grouping by cluster method and costing) the costs of three activities of cacao establishment and annual management in San Vicente de Chucuri and El Carmen de Chucuri (Santander, Colombia) were structured. The grouping phase identified two productive typologies, one with low technification level and another with a medium level; moreover, technification levels were corroborated through cost structuring, in which, for all the activities, the medium technification typology generated higher average values than the low one. General cost structures showed that the most important item is labor, followed by inputs and plant material. The disaggregation of costs into monetary and non-monetary (implicit cost) showed that producers with low technification and low frequency of input use present 35% of non-monetary costs per tree, while producers with some level of crop management and relatively high execution of activities have a slightly higher non-monetary cost, 45% of the average total cost per tree. Within non-monetary costs, the labor (mainly family labor) contributed, on average, to 34% of the total cost structures, which constitutes a risk management factor when defining the financial equilibrium point of the cacao production activity.

Key words: typology of producers, implicit cost, cluster, family labor.

RESUMEN

Con base en una metodología de doble fase (tipificación y costeo), se estructuraron los costos de tres actividades de establecimiento y del manejo anual del cultivo de cacao en los municipios de San Vicente de Chucurí y El Carmen de Chucurí, en el Departamento de Santander, en Colombia. La fase de tipificación arrojó dos tipologías productivas, una con bajo nivel de tecnificación y otra con nivel medio; los niveles de tecnificación fueron corroborados mediante la estructuración de costos, donde, para todas las actividades, la tipología de media tecnificación generó mayores valores promedio que la de baja tecnificación. Las estructuras generales de costos evidenciaron que el rubro de mayor peso es la mano de obra, seguido de insumos y material vegetal. La desagregación de los costos en monetarios y no monetarios (implícitos) arrojó que, los productores con baja tecnificación y baja frecuencia de uso de insumos utilizan un 35% de costos no monetarios por árbol, mientras que los productores con un nivel de manejo de cultivo y de ejecución de actividades relativamente alto tienen un costo no monetario un poco más alto, 45% del costo total promedio por árbol. Dentro de los costos no monetarios, el rubro mano de obra (principalmente familiar) aportó, en promedio, el 34% del total de las estructuras de costos, hecho que se constituye en un factor de gestión de riesgo en la definición del punto de equilibrio financiero de la actividad cacaotera.

Palabras clave: tipologías de productores, costo implícito, clúster, mano de obra familiar.

Introduction

During 2016, Colombia produced 56,163 t of cacao (*Theobroma cacao* L.) in an area of 165,844 ha (FAO, 2018); moreover, the province with the largest cacao producing area at the national level was Santander with 31.6% (DANE, 2016), in farms characterized by being of farmer economy with a long family tradition (Mojica *et al*., 2006). According to the production characteristics mentioned before, the cacao crop fits within the definition of an "agricultural system", in which the emergence of basic resources, the monitoring of business guidelines and family related livelihood means are highlighted (Dixon *et al*., 2001). In this sense, the use of basic resources or production factors (i.e. land, labor and capital), family related or not, generate necessary expenditures to maintain the crop and are defined as production costs (Zugarramurdi *et al*., 1998). The importance of the costs and their adequate registration and interpretation lies

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in the fact that the competitiveness level of the system can be measured and monitored (Perfetti *et al*., 2012).

Maletta (2011), addressing the cost analysis, emphasizes that the coexistence of family and business features within the cacao production system in Colombia generates a differentiation in the amount of real monetary disbursements made during the production process. In accordance with the above mentioned, although family and business production requires the use of labor, family farming uses non-contracted work outside the farm this is rarely remunerated (Schejtman, 2008). In addition to the use of unpaid labor in family farming systems, it is possible to add the use of inputs and their own seeds as non-monetary or implicit disbursements generators (de Renolfi, 2007) or non-monetary costs (Gómez, 1987; Seminario, 2004); this cost category is important within cost structures because it allows a further analysis of their own resources, destined to cacao production, and eventually, the opportunity costs that this task implies (Parkin and Esquivel, 2006). Furthermore, within the financial analysis on the sale prices, non-monetary costs can be the defining factor for the production costs in terms of producer satisfaction (De Janvry, 1995).

According to the aforementioned, the main objective of this study was to show the required cost (monetary and non-monetary) for conducting a start-up and the annual activities to produce cacao. The analysis includes the identification of two typologies or producer groups, one with low technification level and the other with medium level in the municipalities of El Carmen de Chucuri and San Vicente de Chucuri in the province of Santander, Colombia. Our analysis focuses on the required cost for a producer, in the above-mentioned area, to carry out three activities for the crop establishment (new sowing, rehabilitation and renewal) along with the annual maintenance activities. Based on the productive nature of the system in the area, costs were disaggregated into monetary and non-monetary, highlighting quantitative relevance of the activities that do not generate real money disbursement. Furthermore, on average, for the activities analyzed in the two typologies identified, 36% of the costs are classified as implicit or non-monetary, with labor being the most important item, and with an average family contribution of 34% of the total cost.

Materials and methods

Given the productive variability that can be identified in a territory, the process of obtaining the cost of start-up and annual cacao production activities in the province of Santander was divided into two phases; the first one considered the typification or grouping of producers given the technology used, while the second one structured the costs of each group of the identified producers.

Typification of producers or grouping by cluster

In order to determine the typologies of cacao producers in the region, a two-step exploratory sequential analysis (Jokiniemi *et al*., 2018) was performed. During the first step, a workshop with regional cacao experts from FEDECACAO (National Cacao Producers Federation) and AGROSAVIA (Colombian Agricultural Research Corporation) was conducted; these experts described all the activities required for producing cacao in Santander and prioritized those with an important impact in the production cost, such as plant material, the establishing process, and the daily labor conducted by the farmers. Based on the results of the workshop, five variables were identified and used in the second step. In addition, experts concluded that fixed costs are not relevant for this production system in the region; in this sense, the costs considered were not included in the analysis.

The first variable is the type of plant material: within the study area, two types of plant material were identified and due to their management and production characteristics, they generate a differentiation in the production cost patterns. The first material was a cacao hybrid, which results from the sexual crossing of two cocoa trees, guided by specialized operators after a selection process, trying to obtain certain desirable characteristics. The crossing is carried out between clones with different features to improve diverse aspects of interest such as fruit quality, productivity, precocity, response to pests and diseases, among others. The second type of plant material was a clone (the concept does not mean that all plants developed from the same clone are phenotypically identical in all their characteristics) with a uniform genetic material derived from a block and propagated by vegetative means, and in which plant behavior depends on the genotype-environment interaction. Palencia and Mejía (2003) stated that a plant varies in its appearance, production, fruits or beans, among others, according to the climate, soil type, water, diseases and other causes.

The second variable is the type of crop establishment: given that cacao trees are perennial species and usually reduce their productivity under prolonged cycles, duplicated tree establishment mechanisms with productive potential were used as follows: a) New sowings: the establishment of previously grafted trees in nurseries or at the farm; in this case, all tracing and hole digging tasks were needed to be carried out. b) Rehabilitation: a practice that must be carried out on severely damaged trees due to poor pruning management, advanced phenological stage, and plantation abandonment due to low or nule production area in primary branches. For Mejía and Argüello (2000), this is the effect of mechanical damage or unproductive planting material, which constitutes an obstacle to efficient agronomic management execution, especially during harvesting and control of diseases, such as witches' broom (*Moniliophthora perniciosa*) and moniliasis (*Moniliophthora roreri*).

The third variable is the fertilization labor and the application/no application of amendments and/or fertilizers: when the application of a fertilizer or an amendment is carried out, the type used must be stated as chemical, biological, or organic.

The fourth variable identified is plant pruning: pruning practices throughout the year, their quantity and type must be stated, since these can be formative, sanitary or for maintenance purposes.

Finally, the fifth variable is disease management labors: sanitary applications and pruning are practices focused on disease management, such as monilia pod rot and witches' broom (Aime and Phillips-Mora, 2005).

Once the variables were defined, and for the second step, the cluster analysis technique was applied using the statistical package RStudio (National Agricultural Survey ENA, 2015, used as database). According to Villardón (2007), this type of analysis allows the structuring of a data set into groups whose similarities cannot be observed a priori, but once identified, are useful for statistical interpretation; according to the objective of the study data, the selection of the final number of clusters follows a subjective exercise. In this sense, in order to verify the results regarding grouping of producers with the reality of the area, these analises were socialized and discussed with a professional staff from FEDECACAO and AGROSAVIA that had experience in cacao production system management in Santander. From these meetings and by consensus, it was concluded that, in the cacao tree cultivation area of San Vicente del Chucuri and El Carmen del Chucuri, there is a contrast between two types of producers (typologies); those who perform some work to produce cacao, and those who are almost entirely dedicated to harvesting the grain without prior management.

Structuring cacao production costs

This phase of the process was developed with the collection of primary information in 60 cacao-producing farms (convenience sample) in the municipalities of San Vicente del Chucuri and El Carmen del Chucuri in the province of Santander, Colombia. A structured survey was conducted at the farms (with farm owners). The information obtained in the surveys was consolidated in a database, considering the difference for each typology compared to rehabilitation (RH), renewal (RN), new plantings (NP) and annual managment (AM) activities. Each of these activities was divided into items, such as plant material (sexual and asexual seed types), labor and inputs, which in turn, were subdivided into monetary and non-monetary costs.

In order to determine the range of cost values of each of the activities evaluated and to some extent the probability of occurrence of each value which introduces risk analysis to the costing exercise (Palisade Corporation, 2016), outcomes were adjusted to probability distributions using the statistical package @RISK 7.0. The value of the unit cost is COP $/tree, because this form of measurement can offer a better calculation of the cost per hectare considering the specific regional density. This will allow a better decision making and it is considered more approximated because the studied zones do not have the same densities, as it can happen in other areas or crops. In such a way, based on the cost per tree, the probability distribution of the cost per activity and the weight of the non-monetary costs within the productive structure, the difference in prices (in terms of percentage) that can be supported by each typology was established to generate financial returns that cover, at least, the monetary costs.

Results and discussion

Based on the methodology and variables mentioned above, and even though the dispersion diagram and the cluster analysis (Fig. 1) showed three possible clusters, the consensus reached by the experts of FEDECACAO and AGROSAVIA grouped the results as part of only two types of producers: those that perform some tasks to produce cacao and those that are dedicated almost entirely to the harvest of the grain without prior handling.

The first typology is comprised by 45% of the observations collected, in which producers maintain a low technological level compared to cacao management systems. Regarding fertilization and type of application, 86% do not perform any activity focused on this management practice; moreover, 91% do not perform chemical fertilizer applications and only 14% apply some kind of organic fertilizer. Considering phytosanitary management, the levels are equally low, i.e. 100% of the observations showed that cacao producers do not carry out any cultural management of pests and diseases. Furthermore, for 99% of the observations, organic or biological products are not applied, and for 92% of the observations, there is no application of pesticides of chemical synthesis. In relation to pruning, either focused on maintenance or for sanitary purposes, 75% of the observations showed that this practice is not carried out throughout the year. For the case of variables as type of material, crops and municipality where crops are established, there is no marked trend, which allows us to infer that these are not differentiating variables compared to the crop management, and for this reason, are not variables that guide cacao production cost patterns.

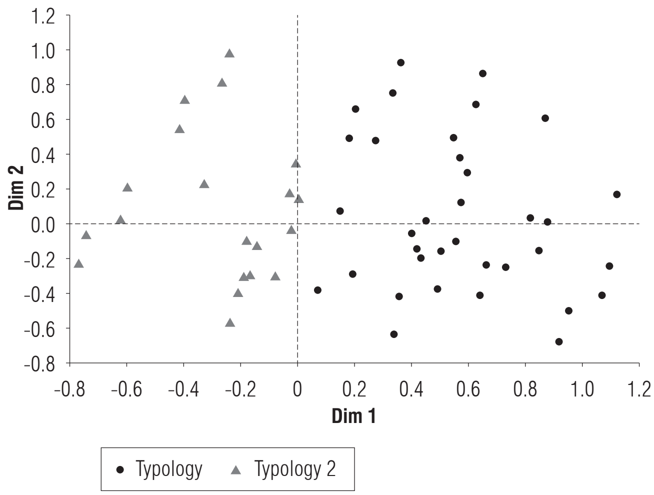

FIGURE 1. Dispersion diagram, typification for cacao productive system in Santander.

Regarding the second typology of producers, 55% of the observations of the samples were atributed to this category, showing a more advanced technological level than those from typology 1 (Fig. 2). In this way, compared to the fertilization variable, 67% of these producers apply chemical fertilizers, while 39% apply organic fertilizers. In the case of phytosanitary management (pests and/or diseases), there is a predominance of chemical management that is carried out in 68% of the properties, followed by cultural management with 37% and biological management with 27%. Regarding maintenance and/or phytosanitary prunings, the observations showed that producers perform them in 53% of the productive units (farms). As in the first typology, the variables of harvest, the type of material, and the location did not generate a trend; a fact that indicates that these variables can not guide the variability of production costs of the cacao crop.

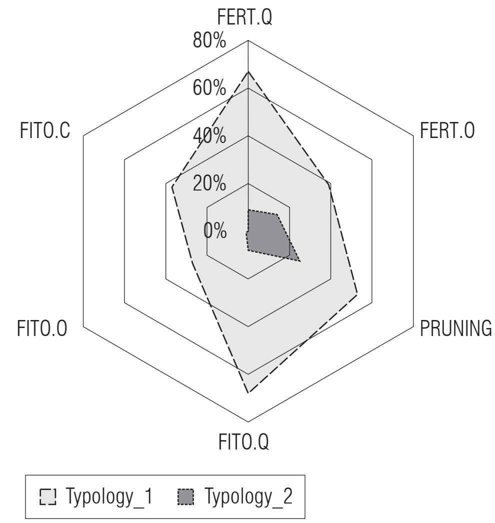

FIGURE 2. Radial diagram of the typologies identified (Fert.Q = Chemical fertilization, Fert.O = Organic fertilization, Fito Q = Phytosanitary management with chemical sources, Fito O = Phytosanitary management with organic sources, Fito C = Phytosanitary management with chemical and organic sources).

The results obtained in the typification phase in the municipalities of El Carmen de Chucuri and San Vicente de Chucuri, in which two groups were identified and differentiated by the intensity level of the productive activities carried out, contrast with what was observed by Mantilla *et al.* (2000). These authors identified that, at a technological level, the municipalities of El Carmen de Chucuri and San Vicente de Chucuri (altogether) have a greater tendency towards the technological management of cacao trees compared to other municipalities in the province. Likewise, the two typologies identified, or at least the one with the highest intensity level of productive activities, differs from the results published by Ramirez Sulvarán *et al.* (2014), in which cacao producers from the municipalities of Norte de Santander were identified as a single typology of mercantile-family characters.

On the other hand, there is agreement between the results observed in our study and the fourth typology described by Purdue University and CIAT (Lundy *et al.*, 2017) in the province of Santander, where the productive level is diversified with different productive levels, in which the improvement has occurred, among other reasons, by the combination of traditional and new productive system practices. Finally, there is concordance between the results observed and the typologies mentioned in a methodological census of cacao carried out by DANE and cited by Superintendencia de Industria y Comercio (2011), in which three levels of technification are mentioned (medium, low and high); it is worth noticing that the first two are similar to those observed in El Carmen de Chucuri and San Vicente de Chucuri.

Costs structure

In order to verify the coherence of cost data with the results of the typification, the cost structures were subjected to a cluster analysis. In this way, the respondents were divided into two groups that were related or not to the execution of the activities of the crop that generate an expense (Fig. 3). The first typology comprises 31 farmers, of which 56% carried out rehabilitation during the survey period (i.e. 5 years), 38% renewed their plant material and 41% established new plantings. On the other hand, 29 farmers were included in the second typology, and from these, 38% carried out rehabilitation activities, 66% renovated their plant material and 10% established new crops. Although global activities or items are similar for both clusters, these items were subdivided into activities that, in turn, served as variables for the analysis of conglomerates, and whose difference originated the final values of the cost structures.

The cluster results showed that the typology 1 spans the cacao producers with low technification and low input use frequency. Moreover, this group showed the least number of activities executed specific to cacao (pruning, fertilization and phytosanitary management). Results of the cost structures show that, within the four cost activities considered, the lowest average value per cacao tree is the annual management activities, followed by rehabilitation, renewal and new plantings (Tab.1).

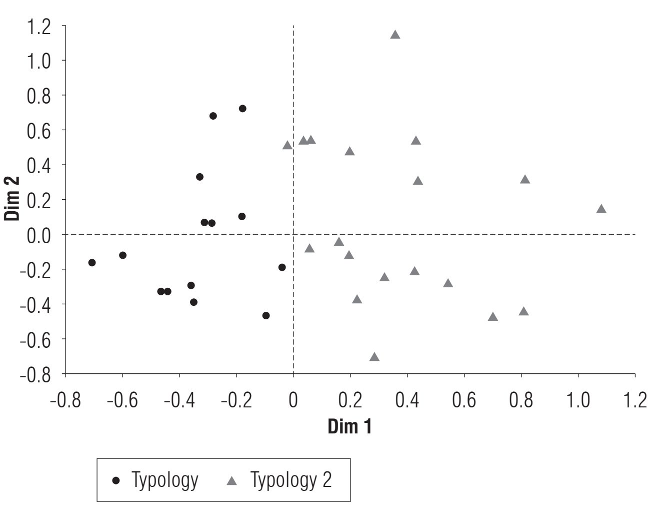

FIGURE 3. Dispersion diagram of costs structure for cacao.

TABLE 1. Average total cost per cacao tree for typology 1 producers.

	RH	RN	NP	AM
Plant material	10%	29%	36%	NA
Labor	81%	60%	56%	93%
Inputs	9%	11%	8%	7%
Average total cost per tree*	$1,998	$2,108	$2,248	$1,901

RH: rehabilitation, RN: renewal, NP: new plantings, AM: annual management.
COP (1 USD is ca. 3,162.40). Representative exchange rate March 11, 2019.

The cost structure for this typology showed relatively high labor values as a trend, showing that the annual management activities (AM) is the highest value with 93% and the new planting activities (NP) is the lowest value with 56%. In all the activities, the use of inputs was relatively low and its weight within the structure does not exceed 10%.

Regarding the monetary costs and those that do not generate real money expenditure (non-monetary), the percentage of what is paid is higher (monetary costs) in all cases (Tab. 2); the percentage values of the non-monetary costs ranged between 29% for renewal (RN) and 48% for SN (rehabilitation (RH) and AM activities have non-monetary costs of 32% and 31%, respectively). According to the above mentioned, under an analysis of disposable income (gross income minus monetary costs), a farmer could find an equilibrium price that is 31% lower than what would be needed under a traditional utility analysis (total income minus total costs).

Furthermore, the results of the four cost activities show differences in the probability distribution that each one possesses (AIC criterion). In the case of rehabilitation, the largest adjustment was made under an ExtValue distribution (Fig. 4A), with distinctive tails to the left and right sides of the mean, with a value of COP$ 1998. This procedure was also performed in the range of values in which 90% of the observations are found from COP$ 1,047 to COP$ 2,958, with a higher probability of finding values below the average (57%). Likewise, the renewal activity -RN generated an exponential distribution (Fig. 4B) with a mean value of COP$ 2,108 and range values between COP$ 547 and COP$ 3,779; although it does not have tail values to the left of the mean, it has a higher value probability in this side of the distribution. Furthermore, new plantings show a uniform distribution (Fig. 4C) in which 90% of the values range between COP$ 1,825 and COP$ 2,670, with an average of COP$ 2,248; in this case, the observed frequency generates the same probability for all values. Finally, the annual management activity costs are distributed under a decreasing triangular function to the right (Fig. 4D) with a

TABLE 2. Monetary and non-monetary cost per cacao tree for typology 1 producers.

	RH		RN		NP		AM	
Monetary cost	68%	$1,359	71%	$1,497	52%	$1,169	69%	$1,312
Non-monetary cost	32%	$639	29%	$611	48%	$1,079	31%	$589
Average total cost per tree*	100%	$1,998	100%	$2,108	100%	$2,248	100%	$1,901

RH: rehabilitation, RN: renewal, NP: new plantings, AM: annual management.
COP (1 USD is ca. 3,162.40). Representative exchange rate March 11, 2019.

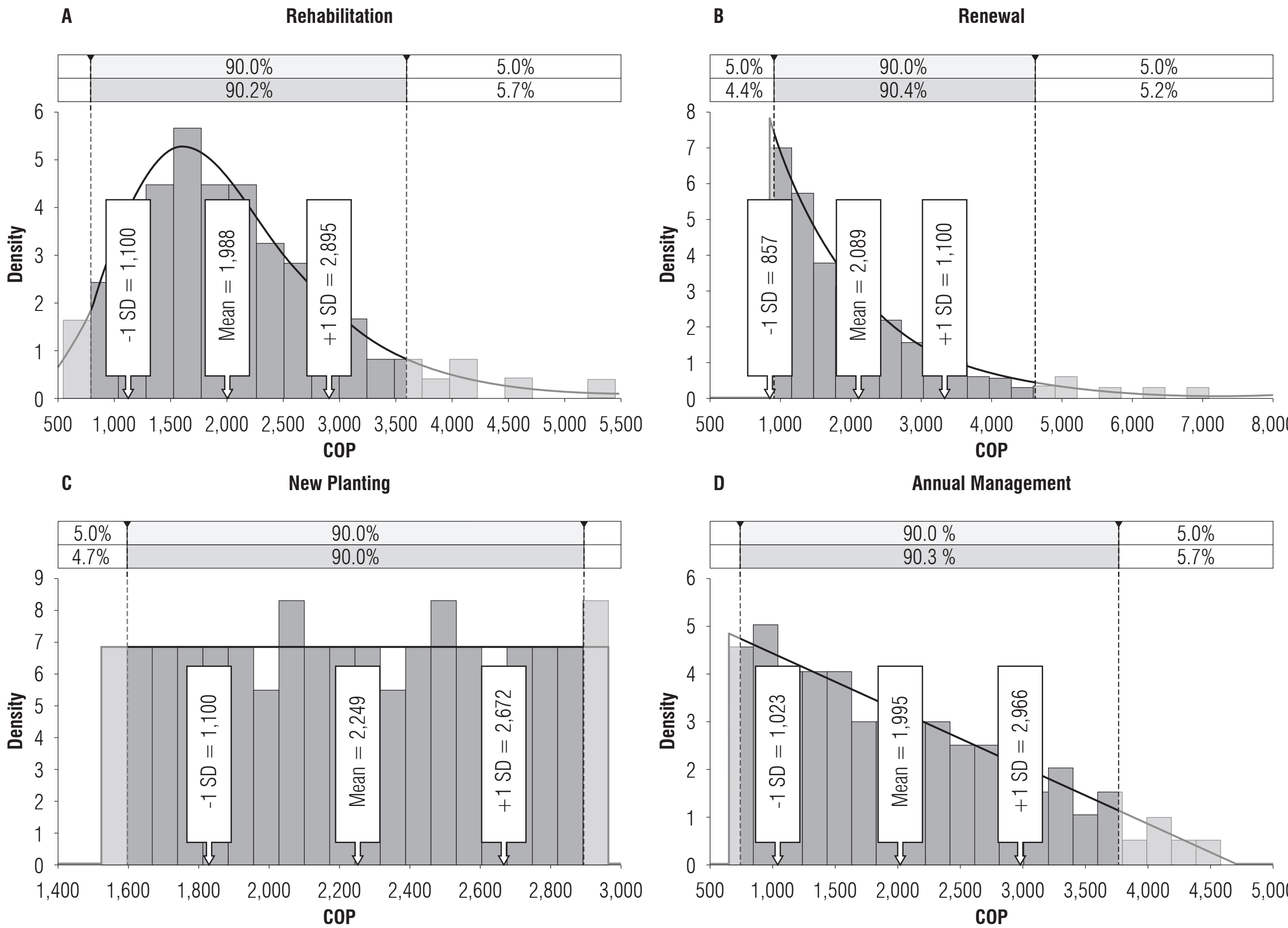

FIGURE 4. Cost distribution for typology 1 producers. SD: standard deviation.

mean value of COP$ 1,901 and a range of 90% of the values between COP$ 896 and COP$ 2,910.

On the other hand, the second cluster shows the typology 2, producers with a relatively high level of crop management and activity execution. In general, among the four cost activities assessed (Fig. 5) the highest average value per tree was reported in the renovation activity with COP$ 2,800, followed by new planting, rehabilitation and annual management activities (Tab. 3).

TABLE 3. Average total cost per cacao tree for typology 2 producers.

	RH	RN	NP	AM
Plant material	14%	27%	38%	NA
Labor	71%	57%	53%	82%
Inputs	15%	16%	9%	18%
Average total cost per tree*	$2,105	$2,840	$2,325	$1,901

RH: rehabilitation, RN: renewal, NP: new plantings, AM: annual management.
COP (1 USD is ca. 3,162.40). Representative exchange rate March 11, 2019.

TABLE 4. Monetary and non-monetary cost per cacao tree for typology 2 producers.

	RH		RN		NP		AM	
Monetary cost	36%	$758	61%	$1,732	55%	$1,279	61%	$1,160
Non-monetary cost	64%	$1,347	39%	$1,108	45%	$1,046	39%	$741
Average total cost per tree*	100%	$2,105	100%	$2,840	100%	$2,325	100%	$1,901

RH: rehabilitation, RN: renewal, NP: new plantings, AM: annual management.
COP (1 USD is ca. 3,162.40). Representative exchange rate March 11, 2019.

As in typology 1, the cost structure of typology 2 showed a trend of relatively high values (over 50% in all cases) in the labor category (Tab. 3). The differentiating factor between the two typologies was shown in the item inputs, which is in all cases, higher in the second typology and confirms what was described in the previous typification.

The relationship between monetary and non-monetary costs was maintained for the RN, NP and AM activities, with a higher percentage of activities with real money disbursement over those that are not remunerated (non-monetary costs); in the case of the RH activity, the proportion was inverted and 64% of unpaid costs were reported with a 36% of monetary costs.

Regarding the weight of the items found in the two typologies, these coincide with those reported by Perfetti *et al.* (2012) in which the labor costs for the cacao management in Santander showed the highest weight percentage within the structure and surpassed the input costs by 54%. Similarly, Barón (2016) reported that for "new plantings", "rehabilitation" and "annual management" activities at the national level, the percentage weight of labor is higher than the percentage value of inputs. In

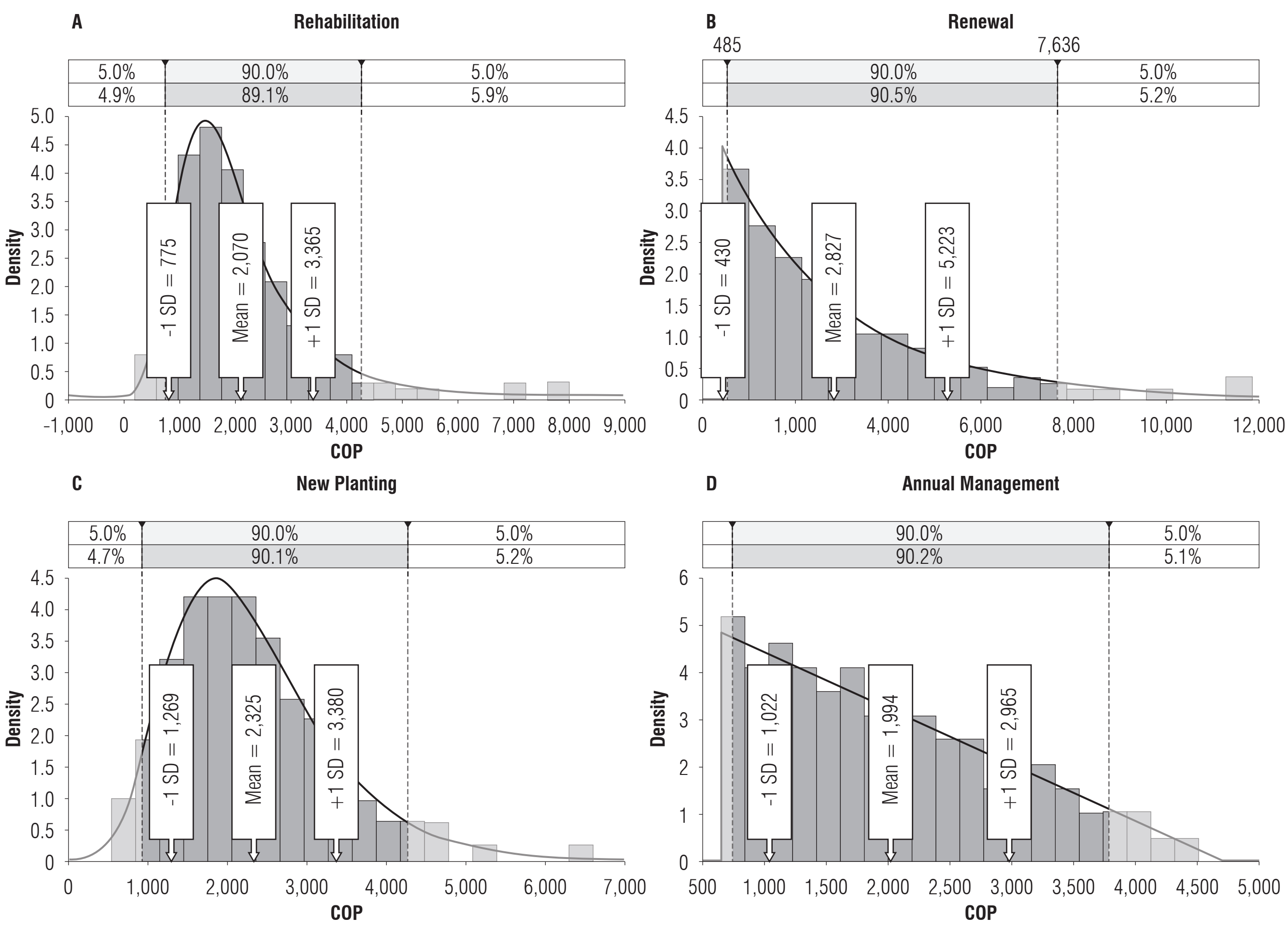

FIGURE 5. Cost distributions for typology 2 producers. SD: standard deviation.

this way, we can conclude that the labor sector is the most important within the cacao cost structure in Santander. Furthermore, Clavijo and Ardila (2015) estimated a production frontier function for cacao, establishing that this variable has a positive and significant effect on the economic efficiency of the crop in the area.

The distributions in which the costs of the activities from typology 2 were adjusted showed a differentiation in frequencies as they did in the first typology. For the case of RH, the largest adjustment (AIC criterion) was found with a LogLogistic function, with queues on both sides of the mean (as in typology 1), the range in which 90% of the data is found ranges from COP$452 to COP $3,820. For the case of RN, the data of typology 2 was adjusted in a similar way as in typology 1 under an exponential function; however, in this case, the average value is the furthest from the Y axis, with a value of COP $2,804. The NP activity data were adjusted under an ExtValue function, with queues at both ends, and high probability of occurrence in values close to the mean (COP $2,325), and 90% of the values lie in the range between $ 1,204 and $ 3,466. Finally, the AM activity, as in typology 1, was adjusted under a triangular function with a most probable value of $ 1,901.

Accordingly, when the comparison between both typologies is made, they showed a differentiation in the distribution of production costs of each of the activities financed. Likewise, weight percentage of each item within the structure showed a marked tendency in which the labor force shows the highest value and the lowest weight is recorded by inputs, while the percentage value is higher for inputs in the typology 2 compared to typology 1. To verify that differences in the variables that gave origin to each typology that generates differentiation in the cost associated to theme, the congregated groups are presented below (Fig. 6).

In general, for all the activities financed (rehabilitation, renewal, new planting and annual management), typology 2 showed higher average values than typology 1, highlighting the renewal activity in which the difference of the mean value of typology 2 compared to typology 1 is COP$ 732; on the other hand, in other activities the difference does not exceed COP$ 107. The lowest average value corresponds to annual management activities in typology 1. Since the data are shown as distribution, it is important to mention that, except for this activity, which has higher mean values in typology 2, distributions have wider ranges, which shows the probability of finding values below the minimum cost in typology 1 in each activity. Based on these results, we can conclude that the cost structures defined for each typology are consistent with the results of the previous typification phase; subsequently, it is inferred that the methodology is useful for cacao productive systems in the demarcated territory.

The contribution of "non-monetary costs" to the cacao cost structure

According to the data previously discussed and summarized in Tables 2 and 4, the importance of the contribution of non-monetary costs to the cacao cost structures in Santander is recognized. In Figure 7, it can be observed that this type of cost varies depending on the type of producer and the item to which reference is made.

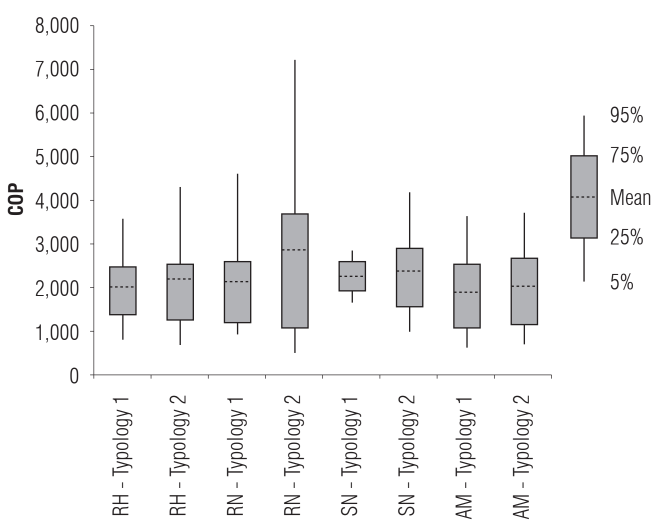

FIGURE 6. Cost distribution of four activities financed by the two typologies. RH: rehabilitation, RN: renewal, SN: new planting, AM: annual management.

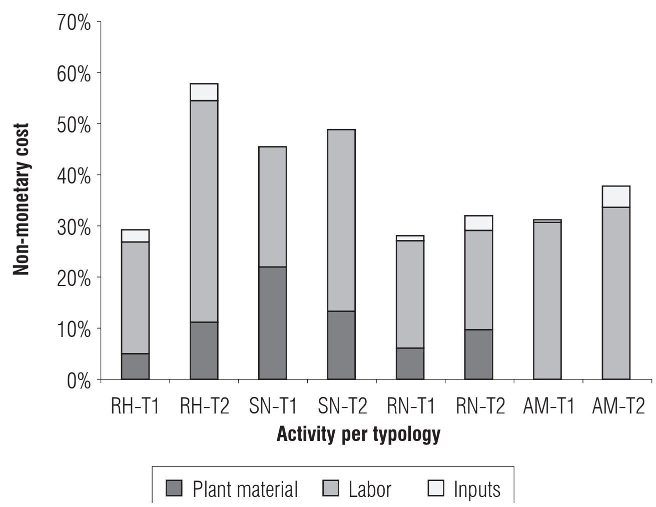

FIGURE 7. Contribution of non-monetary costs to the cacao production cost structures in Santander by per activity. RH: rehabilitation, SN: New plantings, RN: renewal, AM: annual management, T1: Typology 1, T2: Typology 2.

The general contribution of non-monetary costs to the general structure was greater than 28% with an average of 36% at the typology level for both cases and including the four cost activities; the rehabilitation activity in typology 2 reached a value of 58%. The comparison between similar activities in the two typologies, as well as in the average costs analyzed previously, showed a tendency towards superiority in the percentage value of typology 2. In this way, rehabilitation, new planting, renewal and annual management activities showed differences in favor of typology 2 of 28.6%, 3.3%, 3.9% and 6.5%, respectively. At the items level, labor force showed the highest values (28.61% on average) in all activities for the two typologies. The highest labor value was found in the rehabilitation activity in typology 2 with 43%, followed by new planting in typology 2 with 36%, and annual management of this same typology with 34%.

In practical terms, the values of both inputs and plant material (sexual and asexual seeds) that did not represent money disbursement by farmers, can be explained by the campaigns to promote planting that have been developed in the country since 2012 (base year for production cost consultation). These plans established agreements with public and private entities to provide themselves with certified seeds (MADR, 2012). In addition to strengthening capacities on seed issues at the farmer agriculture level, they have shown the physical provision of seeds to farmers as a fundamental component (MADR, 2015). This type of contributions from promotion campaigns generated an average reduction of 9% in the monetary disbursements in the farms surveyed. Moreover, and although the general objective was to increase the national cacao tree cultivation area (MADR, 2012), these campaigns could be framed as an element of social protection that allows poverty reduction (at the family and regional levels) and the promotion of economic growth (FAO, 2015).

On the other hand, non-monetary costs in the labor sector, which averaged 34% in the four activities financed for the two typologies, are related to the cacao production mode in the area and in general; besides, this is also valid in Latin America where family farming is fundamental for the cacao production chains (Arevalo *et al*., 2016). Although family work is not the only feature that characterizes this type of agriculture, it is the main element that can be found in the conceptual definitions of this type of production system (Salcedo *et al*., 2014); in this regard, this type of labor is rarely remunerated and in minimum amounts (Schejtman, 2008). Another fundamental fact in the contribution of unpaid labor (mostly family related), is the low value that opportunity costs represents for farmers when performing tasks within their own farm. This does not necessarily imply a lack of extra-farm employment, understanding that in Colombia the problem is not unemployment per se but low quality and low income (Leibovich *et al*., 2006). According to what we observed in our survey results and based on what was described by Barrett (1996) and IICA (2015), unpaid labor is also a financial and market risk management element for cacao farms of Santander. The nature of the labor contribution reduces the payment for farming activities and the prices necessary to establish an equilibrium point (income = expenditures), when compared to a producer framed in business agriculture in which all inputs and activities are remunerated. The quantitative difference to find this equilibrium point from a cacao producer in Santander is represented by the total production costs (Tabs.1 and 2) minus the non-monetary costs (Fig. 7).

Conclusions

The double-phase methodology (typification- costing) was consistent to obtain the start-up and annual production activities costs for producing cacao in Santander; this was corroborated with the differentiation obtained with the mean cost values (always higher in the typology of medium technification level) that were consistent with what was reported in the typification carried out. We recommend that it should be applied in other cacao producing territories to validate its consistency and generate feedback.

Labor is the most important category within the cost structure of cacao production in Santander. From this we can conclude that this productive system is not intensive in the use of inputs (chemical or organic) or seeds, and that current productivity is based on the work performance in which labor factor is used.

Non-monetary or implicit costs have a considerable weight in both types of producers, for the typology one, the non-monetary cost was 35% of total average cost per tree, while for the second type of producers it represented 47% of their total average cost per tree. This fact confirms the family nature within the cultivation systems in the studied area, and it is an element of risk management for cacao producers in the area. In comparison with industrial farming, family farming has a lower equilibrium price; this difference is equal to the non-monetary cost percentage. Regarding cacao as a national production system, the weight of the non-monetary cost could imply a guide for conducting public policies. For instance, if the traditional family farming system is changed to an industrial one, with paid labor, it could imply a greater risk of financial losses for farmers. In this sense, the policy must be oriented to reduce the unitary production cost. We recommend expanding studies on the behavior of labor in rural territories, addressing an integrated rurality vision.

Acknowledgments

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SCIENTIFIC NOTE

Potential of a *Cladosporium cladosporioides* strain for the control of *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions

Potencial de una cepa de *Cladosporium cladosporioides* para el control de *Tetranychus urticae* Koch (Acari: Tetranychidae) bajo condiciones de laboratorio

Angie Gámez-Guzmán[1], Esperanza Torres-Rojas[1], and Andreas Gaigl[1*]

ABSTRACT

The two spotted spider mite *Tetranychus urticae* is an important polyphagous pest worldwide. It is able to adapt to a wide variety of environments and has a high reproduction rate. In practice, farmers try to reduce losses by using synthetic acaricides. However, frequent and inadequate applications of acaricides have made this mite resistant to many active ingredients, creating the need to search for alternative control strategies. The aim of this research was to identify and to evaluate an indigenous strain of a fungus, which eliminated a *T. urticae* colony in a greenhouse. The isolated fungus was identified through a morphological and molecular characterization as *Cladosporium cladosporioides*. Thereafter, the mites were treated with five concentrations of *C. cladosporioides* conidia ($2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$, and $2x10^8$ conidia ml^{-1}) and a positive control (commercial *Beauveria bassiana* strain, $1x10^6$ conidia ml^{-1}). After 10 d, all treatments achieved at least 50% control; the concentrations $2x10^7$ and $2x10^8$ spores ml^{-1} controlled 73.3% and 81.7%, respectively, surpassing the commercial strain slightly (72.3%). TL_{50} ranged between 5 ($2x10^8$ spores ml^{-1}) and 8 ($2x10^4$ spores ml^{-1}) d, and LC_{50} was $1.95x10^6$. The possible acaricidal effect of this strain on these mites is discussed.

Key words: entomopathogenic fungi, biological control of mites, *Beauveria bassiana*.

RESUMEN

El ácaro bimaculado *Tetranychus urticae* es una plaga polífaga importante a nivel mundial. Es capaz de adaptarse a una amplia variedad de entornos y tiene una alta tasa de reproducción. En la práctica, los agricultores intentan reducir las pérdidas utilizando acaricidas sintéticos. Sin embargo, las aplicaciones frecuentes e inadecuadas de acaricidas han hecho que este ácaro sea resistente a muchos ingredientes activos, lo que genera la necesidad de buscar estrategias de control alternativas. El objetivo de esta investigación fue identificar y evaluar una cepa nativa de un hongo, que erradicó una colonia de *T. urticae* en el invernadero. Se identificó el hongo aislado mediante caracterización morfológica y molecular como *Cladosporium cladosporioides*. Posteriormente, los ácaros se trataron con cinco concentraciones de conidias de *C. cladosporioides* ($2x10^4$, $2x10^5$, $2x10^6$, $2x10^7$ y $2x10^8$ conidias ml^{-1}) y un control positivo (cepa comercial de *Beauveria bassiana*, $1x10^6$ conidias ml^{-1}). Después de 10 d, todos los tratamientos lograron un control de al menos el 50%; las concentraciones de $2x10^7$ y $2x10^8$ esporas ml^{-1} controlaron 73,3% y 81,7%, respectivamente, superando levemente la cepa comercial (72,3%). El TL_{50} varió entre 5 ($2x10^8$ esporas ml^{-1}) y 8 ($2x10^4$ esporas ml^{-1}) d y la LC_{50} fue de $1,95x10^6$. Se discute el posible efecto acaricida de esta cepa para controlar ácaros.

Palabras clave: hongos entomopatógenos, control biológico de ácaros, *Beauveria bassiana*.

Introduction

The efficient control of spider mites has been reported for invertebrate pathogenic fungi (van der Geest *et al*., 2000). The entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuill., *Hirsutella thompsonii* Fischer and *Metarhizium anisopliae* (Metschn.) Sorokin were observed to be promising controls for *T. urticae* (Jeyarani *et al*., 2011). Invertebrate pathogenic fungi are efficient control agents against mites and other arthropods and are increasingly involved in pesticide resistance management in combination with sublethal doses of acaricides (Amjad *et al*., 2012). Moreover, they can be applied in combined strategies, for example, along with the predatory mites *Phytoseiulus longipes* (Amjad *et al*., 2012) or *Neoseiulus californicus* (Oliveira *et al*., 2013).

The breakdown of the *T. urticae* colony in our greenhouse as a result of an entomopathogenic fungal outbreak, apparently from the genus *Cladosporium*, sparked interest in its identification and evaluation of its potential as a biological control agent for this mite. This fungus has been widely

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studied against *T. urticae* (Eken and Hayat, 2009). These authors tested 13 strains of *C. cladosporioides* in a lab bioassay, and the total mortality percentage varied from 50.95 to 74.76% for *T. urticae*, with a LT_{50} range between 2.34-3.9 d. These results led the authors to the conclusion that this fungus is a promising candidate for the biological control of *T. urticae*. Since no studies on *Cladosporium* spp. versus mites are known to have been conducted in Colombia so far, the aim of this study was to test the pathogenicity of these Colombian strains against *T. urticae*.

Materials and methods

Studies were conducted in the Laboratory of Entomology, Faculty of Agricultural Sciences, at the Universidad Nacional de Colombia, Bogota. Deceased mites (*T. urticae*) were collected from bean plants (*Phaseolus vulgaris*, variety Cerinza) established in a greenhouse (mean temperature 22˚C ± 10°C measured using a TFA Indoor Digital Thermo Hygrometer during the entire assay, 80% ± 3% relative humidity and 12:12 h light:darkness). *P. vulgaris* in the vegetative stage (three weeks after germination) served as host plants for the mite colony; the plants were irrigated every three days. In order to avoid the presence of any infested mites, healthy mites were obtained from the Center of Biosystems of the Universidad Tadeo Lozano in Chia (Cundinamarca, Colombia) and from Flores de Tenjo Ltda. (Tenjo, Cundinamarca, Colombia).

Strain isolation and identification

The fungal strain was isolated from dead mites in the colony in the entomology greenhouse of the Faculty of Agricultural Sciences, where an apparently epizootic event occurred in 2010. The fungus was cultured in Potato Dextrose Agar (PDA) medium and kept it in an incubator at 25˚C. This procedure was repeated until monoconidial cultures were obtained.

The fungus was identified by considering its morphological and genetic characteristics. The morphological part was described using a culture grown on a solid PDA medium following the key developed by Bensch *et al*. (2012). For the molecular identification of the fungus, genomic DNA was isolated as follows: (i) to a 2 ml Eppendorf tube containing 1000 µl of lysis buffer (50 mM Tris-HCl [pH 8.0], 100 mM NaCl, 1% Sodium Dodecyl Sulfate, SDS) and 425-600 mm sized acid-washed glass beads (Sigma-Aldrich, St. Louis, USA), a small lump of fresh mycelia was added by using a sterile toothpick. After a quick vortex mix, the Eppendorf tube was left at room temperature for 15 min and centrifuged at 10,000 g for 10 min at 4ºC. (ii) The supernatant was transferred to a 1.5 ml Eppendorf tube, and an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added; the sample was vortexed and centrifuged at 10,000 g for 10 min at 4ºC; this step was repeated using an equal volume of chloroform-isoamyl alcohol (24:1). (iii) After transferring the supernatant to a new 1.5 ml Eppendorf tube, an equal volume of isopropyl alcohol was added and briefly mixed by inversion and stored at -20ºC for at least for 1 h. The sample was centrifuged at 10,000 g for 10 min and the supernatant was discarded. (iv) The resultant DNA pellet was washed in 300 µl of 70% ethanol two times, and the supernatant was discarded each time after the pellet was centrifuged at 10,000 g for 5 min at 4ºC. The DNA pellet was air dried and dissolved in 50 µl of Tris-EDTA and incubated with RNAase (10 µg/ml) at 37ºC for 30 min. Different dilutions (1:1 and 1:5) of the purified DNA were used in 50 µl of the PCR mixture. The internal transcribed spacer region (ITS) from the rRNA was amplified using the ITS 1-4 primers, following the conditions described by Avellaneda-Torres *et al*. (2014). The obtained sequences were analyzed using the software Genius, version PRO 5.1.5., and compared with sequences in the NCBI GenBank using Basic Local Alignment Serach Tool (BLAST) (Altschul *et al.,* 1990; Benson *et al*., 2005).

Preparation of inoculum

The inoculum was produced using pure fungus cultures grown on PDA in sealed Petri dishes and incubated at 25˚C. The spore solutions were prepared by adding 10 ml of distilled and sterile water and 0.1% Tween 20 on the pure culture and scraping the medium with a sterilized needle. This solution was passed through a cloth, and the number of conidia per ml was counted in a Neubauer chamber under a light microscope.

Five different concentrations were prepared from a $2x10^8$ conidia ml^{-1} stock suspension, which were diluted in water at $2x10^4$, $2x10^5$, $2x10^6$, and $2x10^7$ conidia ml^{-1} for immediate application in the bioassay.

Bioassays under controlled conditions

Humid cotton was placed in a Petri dish and was covered with an upside-down rose leaf (variety Charlotte grafted on the Natal Briar rootstock). Then, 20 mites were placed on each leaf (experimental unit). Every experimental unit was repeated five times. The fungus was applied using a mobile sprayer located in the weed greenhouse of the Faculty of Agricultural Sciences. First, the sprayer was calibrated as follows: 1 kg/cm^2 pressure, 0.48 m s^{-1} speed and 0.57 l min^{-1} as volume. After spraying the Petri dishes, they were placed in an incubator (25˚C, 80 % RH, 12:12 h light:darkness) for 10 d.

The seven treatments were conducted as follows: T1: negative control (water and Tween 20 0.05 %), T2: positive control applying the commercial product Bassianil®, with *Beauveria bassiana* as the active ingredient (Perkins Ltd., Palmira, Colombia) at a concentration of $1x10^6$ conidia ml^{-1}. Based on previous experiments and the study by Saranya *et al.* (2010), six conidia concentrations were selected; the treatments (fungus concentrations = conidia ml^{-1}) were T3: $2x10^4$, T4: $2x10^5$, T5: $2x10^6$, T6: $2x10^7$, T7: $2x10^8$. The entire experiment was repeated three times over time. The mites were counted for 10 d, starting on the first day after application, and the accumulated percentage of dead individuals was calculated.

Statistical analysis

Abbott's equation (1925) was used in order to correct the mortality data. The effects of treatments on mite mortality were compared using the general linear model procedure (PROC GLM, SAS Institute 2013); significant differences between the treatments were identified with Tukey's test HSD (P<0.05). The treatments were arranged using a completely randomized design.

Results and discussion

Strain identification

The isolated strain taken from the PDA cultured fungus presented a brownish dark color surface, a black color reverse; the plain surface was haired and occasionally turned to dust. At the microscopic level, we identified hyphae septate, branched and dark (data not shown). These results are corroborated by the observations of Bensch *et al.* (2012), who described the surface ornamentation of conidia in the C. *cladosporioides* complex as quite variable, ranging from smooth, or almost so, to irregularly verruculose-rugose, verrucose or rough-walled in some species.

The extracted DNA was visualized with agarose gel electrophoresis (Fig. 1A), and the size of the successfully amplified sequence, using ITS1-4 primers, was about 513 base pairs (Fig. 1B). After the sequencing and DNA data comparison using BLAST search, highly homologous sequences were obtained with GeneBank samples in NCBI. This indicated that the microorganism corresponds to the fungus of the division Ascomycota, subphylum Pezizomycotina, order Capnodiales, family Cladosporiaceae, genus *Cladosporium*, and species *Cladosporioides*. Its coverage was 100%, the E-value was 0.0, and the identity was 100% (accession number LN808903.1).

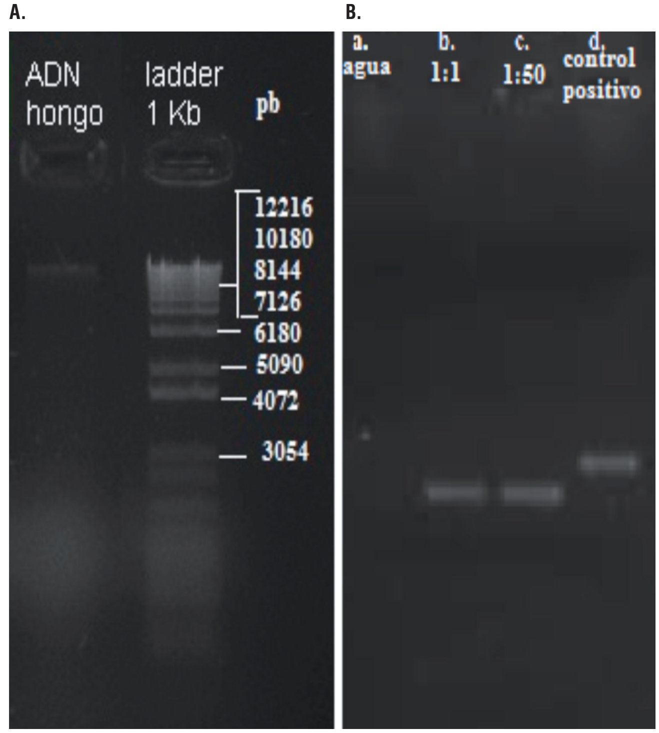

FIGURE 1. Agarose gel analysis of DNA extracted from a fungus strain (A) and Gel electrophoresis of amplicons products with the primers ITS1-4 of the rRNA gene on purified genomic DNA (B). Amplicons (513 bp) from 1:1 (b) and 1:50 (c) ADN dilutions; NC: negative control; no DNA (a). PC: positive control (d). MM: 1 Kb DNA molecular weight marker; DNA lambda (Thermo).

Cladosporium spp. These species are ubiquitous, saprobic, dematiaceous fungi and have been associated with human and animal opportunistic infections (Sandoval-Denis *et al.*, 2015). Their entomopathogenic potential has been described since the early eighties, when their pathogenicity to insects was recorded (Samways and Grech, 1986). Nowadays, *Cladosporium* spp. are considered effective biological control agents against insects and mites at the laboratory level (Sosa-Gomez *et al.*, 2010; Bahar *et al.*, 2011). Nevertheless, among the arthropod-associated ascomycete fungi, uncertainties remain about the extent to which species in ubiquitous genera, such as *Cladosporium*, *Aspergillus*, *Penicillium*, and *Fusarium,* are pathogenic to arthropods or might be opportunistic secondary pathogens or saprotrophics that colonize available cadavers (Samways and Grech, 1986). Thus, histopathologic studies are necessary in order to prove whether this organism is the causal agent of an arthropod's death.

Entomopathogenic activity of *Cladosporium cladosporioides*

It was observed that the dead mites presented a brown or dark green color resulting from the attack of the fungus. When the fungus entered an advanced stage of development, the color turned black. Table 1 shows significant differences between the different conidia concentrations for all days (Tukey's test, $P<0.05$). The concentration $2x10^4$ conidia ml^{-1} achieved moderate control of the mites, 62%. The highest mortality (81.7%) was obtained with $2x10^8$ conidia ml^{-1}, suggesting that this fungus might be a promising candidate for the control of mites and should be evaluated under field conditions.

These results surpassed the efficacy of the commercial *B. bassiana* strain (72.3 %). The negative control (only water + Tween) showed a mortality of 20.7%, which was similar to the results obtained by Chandler *et al*. (2005). They hypothesized that a high mortality in the control might be associated with an interaction between fungus and non-fungus mortality factors in a treatment bioassay; for instance, lesions caused by transferring the mites to the experiment unit, a short life cycle and a non-uniform age of the mites.

Table 1 gives evidence for the progressive activity of the fungus: the concentration $2x10^8$ ml^{-1} caused a 50% mortality after 5 d, $2x10^7$ ml^{-1} after six, $2x10^6$ ml^{-1} after seven and the treatments with the lowest concentration ($2x10^5$ ml^{-1} and $2x10^4$ ml^{-1}) caused 50% mortality after 8 d. A similar mortality rate was reported by Gatarayiha (2009), who observed that *B. bassiana* caused a lethal time, $LT_{50,}$ between 5.5 and 8.9 d. Unfortunately, it is impossible to compare our results with other reports because of different experimental designs of bioassays with different fungi and arthropods (Chandler *et al*., 2005; Bahar *et al*., 2011). However, other criteria for fungi selection should be considered, such as a high conidia production and high final mortality rates (Chandler *et al*., 2005; Bahar *et al*., 2011). Lower conidia concentrations ($2x10^4$ and $2x10^5$ conidia ml^{-1}) are able to reduce mite populations. However, higher concentrations ($2x10^6$, $2x10^7$ and $2x10^8$ conidia ml^{-1}) definitely shorten the lethal period, which was corroborated by our experiments.

A mean lethal concentration LC_{50} of $1.95x10^6$ conidia ml^{-1} was found. This concentration was low compared with the LC_{50} of other fungi, such as *B. bassiana* ($8.65x10^7$ conidia ml^{-1}), *Hirsutella thompsoni* ($1.06x10^8$ conidia ml^{-1}), and *M. anisopliae* ($2.43x10^7$ conidia ml^{-1}), on *T. urticae* (Chandler *et al*., 2005). Our results indicate that the fungus showed a good control potential towards mites. It is possible that the fungus also acted through toxic bioactive metabolites, increasing the effect with a typical mycoparasitic infection (Bensaci *et al*., 2015). Samways and Grech (1986) stated that *Cladosporium oxysporum* is capable of inducing major decreases in the aphids *Toxptera cictriculus* and *T. erytreae*. Moreover, Shaker *et al*. (2019) provided support for this hypothesis through the isolation of two major compounds from *C. cladosporioides*, which were identified as 3-phenyl propanoic acid and 3-(4β-hydroxy-6-pyranonyl)-5-isopropylpyrrolidin-2-one. These compounds caused 100% mortality for the aphid *Aphis gossypii*.

The toxic properties and degree of their toxicity vary depending on the administration, chemical structure and concentration (Piecková and Jesenská, 1999). Since *Cladosporium* spp. form tiny conidia in high amounts (Piecková and Jesenská, 1999), it can be concluded that this fungus is

TABLE 1. Percentage of corrected mortality (Abbott, 1925) of *Tetranychus urticae* after applications of *Cladosporium cladosporioides* over 10 days.

DAA	Control	$2x10^4$	$2x10^5$	$2x10^6$	$2x10^7$	$2x10^8$	*B. bassiana* $2x10^6$	F_{value}	P_{value}
1	1.3 ± 0.75 c	6.7 ± 4.6 b	9.0 ± 0.9 b	14.3 ± 1.5 a	14.3 ± 1.9 a	16.7 ± 3.3 a	6.7 ± 3.1 b	21.5	<0. 0001
2	2.3 ± 0.9 d	11.7 ± 2.6 c	14.3 ± 1.9 bc	20.0 ± 3.1 ab	23.7 ± 6.1 a	27.0 ± 3.6 a	14.7 ± 4.3 bc	26.0	<0. 0001
3	3.7 ± 0.75 e	16.0 ± 3.0 d	20.3 ± 4.3 cd	27.0 ± 5.2 abc	32.3 ± 7.5 ab	35.0 ± 3.1 a	24.0 ± 3.5 bcd	29.6	<0. 0001
4	5.0 ± 1.2 d	22.0 ± 3.2 c	27.3 ± 4.9 bc	32.7 ± 6.5 abc	40.7 ± 10.9 a	42.3 ± 2.5 a	34.3 ± 7.0 ab	22.7	<0. 0001
5	7.7 ± 1.5 e	28.7 ± 5.7 d	32.3 ± 5.1 cd	37.3 ± 6.9 bcd	47.3 ± 12.4 ab	51.7 ± 3.3 a	44.3 ± 8.9 abc	21.6	<0. 0001
6	9.0 ± 0.9 c	36.0 ± 6.3 b	42.3 ± 8.0 ab	44.3 ± 6.0 ab	54.0 ± 13.0 a	57.3 ± 6.8 a	53.0 ± 6.5 a	23.9	<0. 0001
7	11.3 ± 1.8 c	43.7 ± 8.3 b	48.3 ± 8.7 ab	52.0 ± 6.6 ab	60.0 ± 10.5 a	62.3 ± 5.4 a	58.3 ± 4.9 a	30.2	<0. 0001
8	14.3 ± 1.9 c	51.7 ± 10.1 b	54.0 ± 9.5 b	59.0 ± 4.8 ab	63.7 ± 8.4 ab	69.0 ± 6.9 a	65.0 ± 5.4 ab	32.5	<0. 0001
9	19.0 ± 3.8 c	59.0 ± 8.5 b	57.7 ± 8.2 b	63.0 ± 5.7 ab	68.0 ± 7.8 ab	75.0 ± 8.3 a	68.0 ± 6.5 ab	32.9	<0. 0001
10	20.7 ± 4.9 c	62.0 ± 9.8 b	63.0 ± 7.3 b	70.3 ± 5.4 ab	73.3 ± 7.0 ab	81.7 ± 6.1 a	72.3 ± 6.0 ab	42.4	<0. 0001

DAA: Days after application. *Means followed by the same letter are not significantly different (Tukey HSD, $P\leq0.05$).

an efficient candidate for the biological control of arthropods. Singh *et al.* (2016) described the potential of toxins produced by *Cladosporium velox* for exhibiting activities of Alpha glucosidase inhibitors, affecting the digestion and moulting of *Spodoptera litura*.

It was observed that the LT_{50} of the mites oscillated between seven and nine days when treated with *Cladosporium* and *B. bassiana*, which was significantly different from the control. The lowest LC_{50} ($1.11x10^6$ conidia ml^{-1}) occurred on d 10. The rate of mortality decreased during the entire observation period, whereas the standard deviation increased. Despite the fact that the differences between the commercial strains and our isolate were not statistically significant because of the high mortality in the control. The results suggest that our tested strain may be a promising candidate for biological control. Another experiment under controlled conditions and with higher conidia concentrations verified under semi-controlled and field conditions may give further evidence of the efficacy of this strain as a control agent of mites. Moreover, further studies on secondary metabolites of this strain and their toxicity to tetranychid mites might create options for biological control strategies against this pest. For example, Shaker *et al.* (2019) successfully extracted ethyl acetate from *C. cladosporioides,* leading to 100% control of aphids.

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SCIENTIFIC NOTE

Global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley, Mexico

El calentamiento está reduciendo la capacidad de macollamiento y el rendimiento de grano en trigo en el Valle del Yaqui, México

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ABSTRACT

The effect of temperature variations recorded in eight meteorological stations in Yaqui Valley, Sonora, Mexico, on the tillering capacity and grain yield of wheat variety CIRNO C2008 in the growing cycles December 2016-May 2017 and December 2017-May 2018 was studied. In one of the sites, the crop canopy temperature was increased by +2°C with a T-FACE system (warming) based on the temperature recorded in the nearest meteorological station. With the two experimental variants, the abscisic (ABA) and gibberellic (GA) acid hormones were determined during tillering (initial tillering: 30 d after emergence and final tillering: 45 d after emergence) to explain their contribution to the tillering capacity response. A temperature variability of 1°C was observed in the cycle December 2017-May 2018, as compared to the previous cycle and between the evaluated sites. As a result of the temperature increase effect, the tiller number was significantly reduced. The experimental warming caused a highly significant decrease in the ABA content and an increase in the GA. The temperature variation found in Yaqui Valley had a negative and significant correlation with the grain yield in both experiment crop cycles, which demonstrated that global warming is reducing the tillering capacity and grain yield of wheat in Yaqui Valley.

Key words: climate change, gibberellic acid, abscisic acid.

RESUMEN

Se estudió el efecto de las variaciones de temperatura registradas en ocho estaciones meteorológicas del Valle del Yaqui, Sonora, México, en la capacidad de macollamiento y el rendimiento de grano de la variedad de trigo CIRNO C2008, en dos ciclos de cultivo. En uno de los sitios, la temperatura del dosel del cultivo se incrementó en 2°C por medio de un sistema T-FACE (calentamiento) usando como control la temperatura registrada en la estación meteorológica más cercana. En estos dos tratamientos, se determinaron las hormonas ABA y GA al inicio y final del macollamiento para explicar su contribución a la capacidad de macollamiento. Como resultado, se encontró una variabilidad de temperatura de 1°C en el ciclo de diciembre de 2017 a mayo de 2018 con respecto al anterior y también entre los sitios evaluados. Por efecto del aumento de la temperatura disminuyó el número de hijos. El contenido de ABA disminuyó por efecto del calor mientras que el de GA aumentó. La variación de temperatura encontrada en el Valle del Yaqui correlacionó negativamente con el rendimiento de grano en ambos ciclos de cultivos, demostrando que el calentamiento está reduciendo la capacidad de macollamiento y el rendimiento del grano de trigo en el Valle del Yaqui.

Palabras clave: cambio climático, ácido giberélico, ácido abscísico.

Introduction

High temperatures cause considerable changes in plant morphology, cellular dynamism and hormonal relationships, which modify growth patterns (Argentel *et al.*, 2017), creating heat stress when plants do not overcome the minimum tolerance index (Mahalingam, 2015). A single heat shock during growth may cause irreversible damage to plant physiology and yield (Valluru *et al.*, 2017; Garatuza *et al.*, 2018). Many species, such as wheat, need cold hours (also called heat units) to complete critical phenophases (Ganem *et al.*, 2014); for example, at least 300 cold hours are needed for good tillering. The tillering capacity is directly correlated with yield; however, both depend on temperature (Argentel *et al.*, 2017). The tillering phenophase is hormonally regulated by the relationship between abscisic (ABA) and gibberellic (AG) acids, with temperature variations being the abiotic factor that contributes most to hormonal imbalances (Xie *et al.*, 2016). Currently, in some high producing regions,

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such as Yaqui Valley, Sonora, Mexico, where more than 39% of the domestic wheat production occurs, significant changes in temperature patterns are viewed as evidence of climate change (Lares *et al.,* 2016). This phenomenon, according to parametrized models, will limit the tillering capacity and, consequently, modify the genetic-productive expression potential of wheat (Argentel *et al.,* 2017). For this reason, the present study aimed to evaluate the effect of temperature variability on the tillering capacity and grain yield in eight representative sites in Yaqui Valley during two crop cycles, comparing them to that obtained with an experimental warming of +2°C in the ambient canopy temperature (Garatuza *et al.,* 2018) using the CIRNO C2008 wheat variety.

Material and methods

Experiment area location

The experiment was carried out during the growing seasons of 2016-2017 and 2017-2018 (December-May) under field conditions using a calculus surface of one ha in seven representative sites in Yaqui Valley, Sonora, Mexico, where agro-meteorological stations belonging to the INIFAP (Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias) are located (Fig. 1). These stations record the data of agroclimatic variables, mainly the temperature of the region.

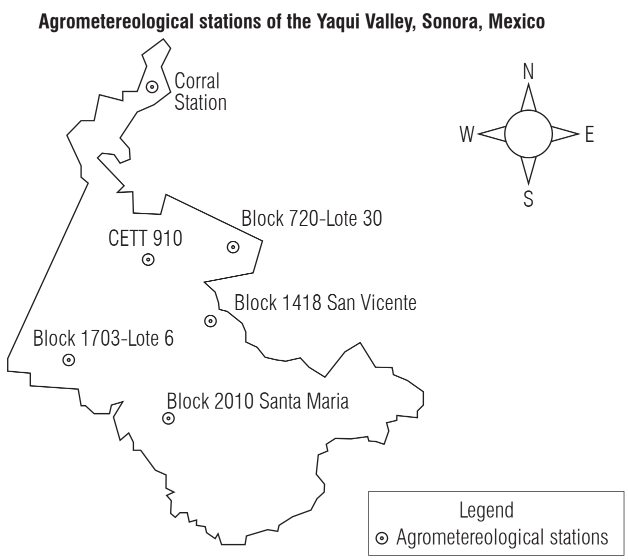

FIGURE 1. Distribution of experimental sites and their respective meteorological stations in Yaqui Valley.

To simulate a site with the maximum temperature (+2°C) predicted for this region, the canopy temperature was increased +2°C with respect to the ambient canopy temperature, at one site in order to evaluate the effect of global warming on the tillering capacity, yield components, and hormonal concentrations during the tillering phenophase. The experimental warming was carried out at the Experimental Technology Transfer Center (CETT-910) of the Instituto Tecnologico de Sonora (ITSON) located in Yaqui Valley at: 27°22'0.4" N and 109°54'50.6" W (UTM: 607393.24 m E; 3027508.34 m N), which is a representative place for wheat crops in Yaqui Valley.

Temperature control

The temperature database was supplied by seven climatological stations located in the experiment sites. The digital memory of the meteorological station records readings every 10 min and provides integrated data by hour and day, averaging the temperature and calculating the thermal sum (heat hours, cold hours) with the maximum and minimum threshold temperature of 10°C and 0°C, respectively, following the methodology of thermal sums of Confalone and Navarro (1999). These data are available at http://www.siafeson.com/remas/index.php.

For the maximum predicted temperature value for the region (warming), a T-FACE system was used (Kimball, 2015), which consisted of raising the crop canopy temperature by 2°C using six thermal radiators per plot (FTE-1000 model, 1000W, 240 V, 245 mm long x 60 mm wide, built by Mor Electric Company Heating Association Inc. Comstock Park, MI, USA) on eight equilateral triangular structures, with sides of 5.2 m. Two radiators were installed on each side of the triangular structures, forming a hexagon, which raised the temperature in a 3 m diameter circle for each plot. To control the temperature, infrared temperature sensors (IRTS Apogee Instruments Inc., Logan, UT, USA) were pointed to each plot at an inclination degree of 45° from the soil horizontal surface, covering a circle with a r = 1.5 m at the center of each plot (Garatuza *et al.,* 2018). The IRTS were registered in a datalogger (CR1000 Campbell Sci, Inc. Logan, UT, USA), which sent a voltage signal to an interface (MAI-05V, Avatar Instruments).

Variety, seeding and crop management

In all sites, the CIRNO C2008 wheat variety was used. This variety is classified as crystalline or hard wheat. It originated from selection in segregating populations of SOOTY-9 / RASCON-37 // CAMAYO crossbreed, carried out at the International Center of Maize and Wheat Improvement (CIMMYT). This variety was released for cropping in 2008 and is widely used in Mexico, particularly in the northwest. The grain yield has reached 5.6 t ha^{-1} and 6.3 t ha^{-1} with two and three irrigations, respectively, at an irrigation rate of 14 cm when this variety was released in Sonora. This variety, after eight years of being released for crops in southern Sonora, has shown a high yield genetic stability (Argentel *et al.,* 2018).

The seeding was done with a sowing machine (SUB-24) on 8-14 December 2016 and 7-14 December 2016 on vertisol soils (Bockheim *et al.,* 2014) with a seeding density of 170 kg ha^{-1}. The background fertilization, in both experiment crop cycles, was done with a standard of 250 kg ha^{-1} of Urea + 100 kg ha^{-1} of monoammonium phosphate fertilizer (MAP, 11-52-00). Three irrigation treatments were applied during the crop cycle. Nitrogen fertilizer was also applied at a dose of 50 kg ha^{-1} of Urea during the first and second irrigation treatments (growth and tillering phenophases). All irrigation treatments were applied with an average water depth of 14 cm and an irrigation interval of 25 d.

During both crop cycles, the presence of the foliage aphid (*Schizaphis graminum*) was observed, and the Muralla Max® pesticide (a.i. Imidacloprid + Betaciflutrin) was applied at a rate of 0.20 L ha^{-1} in the tillering phenophase. Also, a slight presence of broadleaf weeds was observed, which were controlled manually before the irrigation treatments.

Evaluated variables

The tillering capacity was evaluated according to the number of tillers around a principal plant. The final tiller count was done at 45 d after germination.

ABA and GA concentration at the site of maximum temperature (Warming treatment)

For the ABA and GA determination at 15 and 30 d after germination, the methodology of Ortiz *et al.* (2001) was used. Leaf tissue (10 g fresh weight) was collected and immediately frozen in liquid nitrogen. The frozen leaf tissue was freeze-dried for 48 h, ground and extracted in distilled deionized water with an extraction ratio of 1:40 (dry weight: mL water) at 4°C. The ABA and GA concentrations of the extract were determined using high performance liquid chromatography (Wang *et al.,* 2016).

Grain yield

The grain yield (t ha^{-1}) was determined at each experiment site based on a square meter using 16 replicates.

Statistical analysis

For the tillering capacity and grain yield variables, the mean and standard deviation of each treatment (experiment sites) were determined. The differences between the sites were detected with analysis of variance based on a randomized effect linear model (Fisher, 1937). Additionally, a Tukey post-hoc test for $P<0.05$ and $P<0.01$ was applied (Tukey, 1960). To analyze the temperature effect on the grain yield, simple regression was used. The hormonal concentration was compared using a parametric theoretical distribution for t-student quantitative continuous variables (Gosset, 1917) using 16 samples per treatment. For all data analyses, the professional statistical software STATISTICA 11.2 (StatSoft, 2008) was used.

Results and discusion

Wide temperature variability between the studied sites was observed, with a 1°C difference in 2017-18, as compared to the previous cycle. This is an important result that demonstrated a temperature increase in Yaqui Valley that evidences climate change. The site effect explained 95% of the temperature variation according to the randomized effect linear model used for the analysis of variance, while only 2% of the total variability was explained by crop cycle effect. There was not significant interaction between the sites and crop cycles (Fig. 2).

Some reports state that significant variation and increases (about 2°C) have been predicted for several latitudes for the next 50 years, including this area (the semiarid region of Mexico) (Garatuza-Payan *et al.,* 2018). However, in the last crop cycle, the total variability was 1°C.

The tillering capacity experienced significant variation between the sites in both crop cycles, with a reduction in all sites as a result of the temperature variation (Fig. 3).

The hormonal relationship was significantly affected by the warming effect, which shows that temperature increases favor GA synthesis and inhibit ABA synthesis; this hormonal response limited the tillering capacity (Fig. 4).

Some studies have explained that tillering cessation and number of total tillers is regulated by many biochemical, physiological, genetic and environmental factors (Xu *et al.,* 2016). Phytotechnical factors, such as plant density (through an increase in seed density during sowing), can also affect the total tiller number (Pinto *et al.,* 2017).

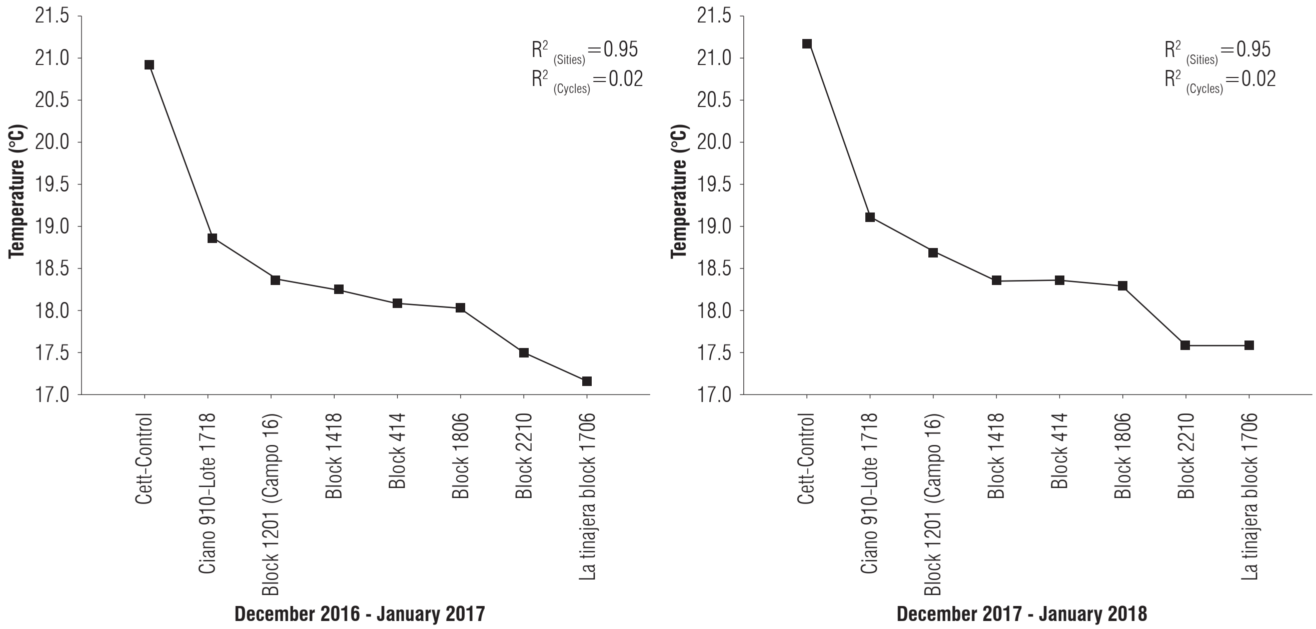

FIGURE 2. Temperature variability at eight experiment sites in Yaqui Valley during the crop cycles December 2016 - January 2017 and December 2017 - January 2018 (tillering phenophase of wheat). R^2: coefficient of determination without adjustment for the sites and crop cycles. Squares represent mean ± standard deviation.

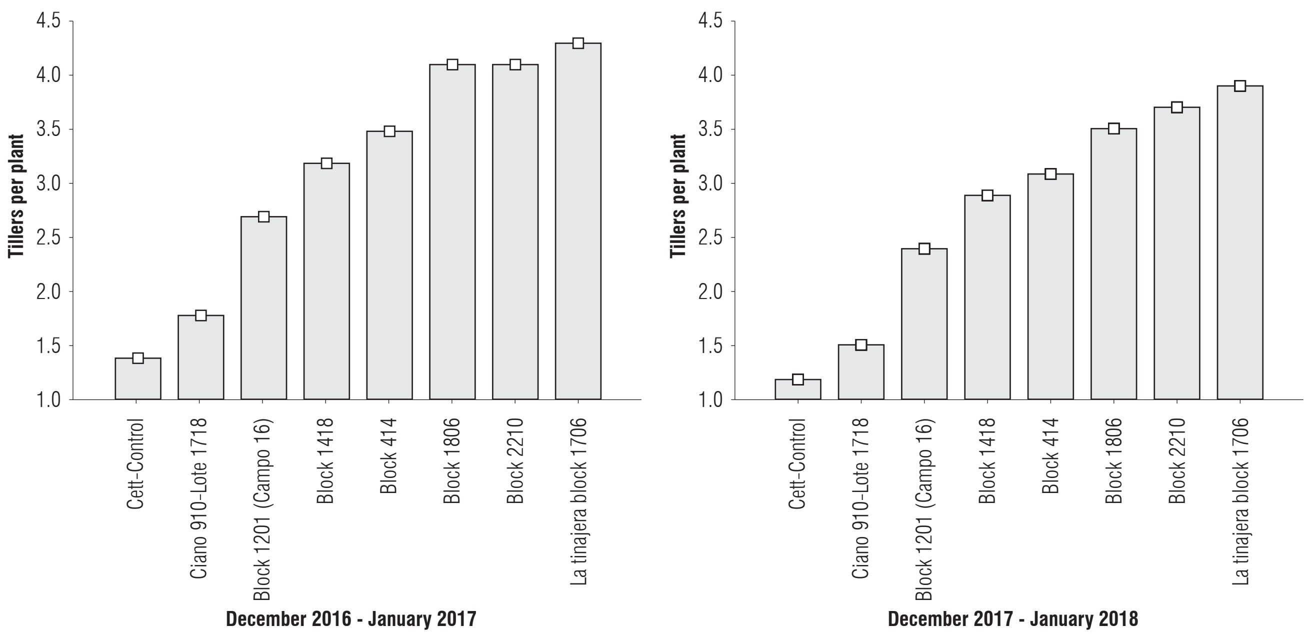

FIGURE 3. Tillering capacity in the evaluated sites in Yaqui Valley during the 2016-17 and 2017-18 crop cycles: A. December 2016 to January 2017, and B. December 2017 to January 2018. R^2: coefficient of determination without adjustment for the sites = 0.96, and crop cycles = 0.04. CV: coefficient of variation for sites = 8.32, and for crop cycles = 1.46. Squares represent mean ± standard deviation.

The tillering capacity in wheat determines plant canopy size (Sattar *et al.*, 2015), photosynthetic area and, more importantly, the number of spikes and full grains per spike (Garatuza *et al.*, 2018).

During the tillering phenophases, there were highly significant differences in the amount of cold hours between the evaluated sites and crop cycles (P = 0.015); CETT-910 (experimental warming) had the least cold hours.

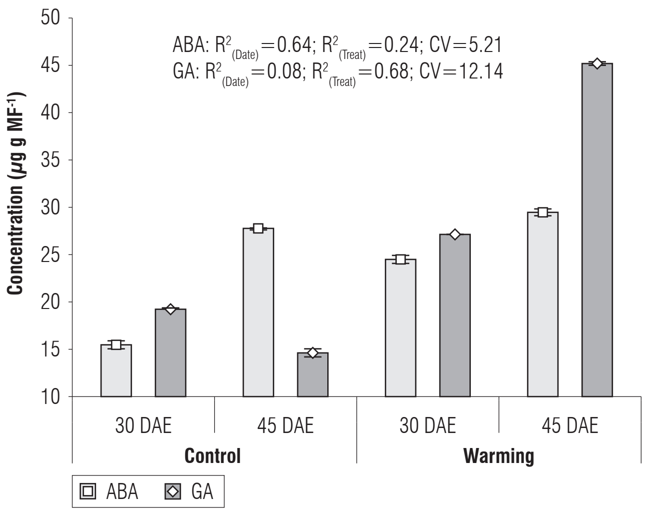

FIGURE 4. ABA and GA content at 30 and 45 d after emergence (tillering phenophases) evaluated in the higher temperature site (crop canopy +2°C) and control site in Yaqui Valley (mean of both 2016-2017 and 2017-2018 crop cycles). R^2: coefficient of determination without adjustment for the phenophases and treatments; CV: coefficient of variation.

Nevertheless, a reduction greater than 30% was observed in cycle 2017-18 with respect to 2016-17. The site effect contributed to 78% of the total variability in the cold hours; however, the crop cycles contributed only 18%. A significant interaction between the sites and crop cycles effects was observed (P = 0.002), which contributed 4% to the total variability (Fig. 5). These results, according to Espinosa *et al.* (2018), demonstrated that the tillering capacity was reduced by the reduction of cold hours in both evaluated crop cycles.

Historically, it has been shown that between 300 and 850 cold hours per season provide a yield near the genetic-productive potential of the varieties, with the greatest contribution to grain yield seen during the first stages, until the tillering phenophase (Zou *et al.,* 2017). Currently, the number of cold hours or heat units is the basis of research in various regions around the world under the context of climate change (Kaur and Kaur, 2017). These studies focus mainly on gene identification and introgression for adaptability to cold hour reductions and on the achievement of hormonal stability during initial growth until the tillering phenophase (Pinto *et al.,* 2017).

Grain yield

The temperature variation showed a negative and significative correlation with grain yield in both experiment crop cycles (Fig. 6). The observed general yield decrease was fit at 92% with the linear model used for the statistical process in the crop cycle 2016-17 and at 51% in the second crop cycle.

Mean temperature values of 18.4°C and 18.66°C were observed during crop phenology in the 2016-17 and 2017-18 crop cycles, respectively. The regression equations were

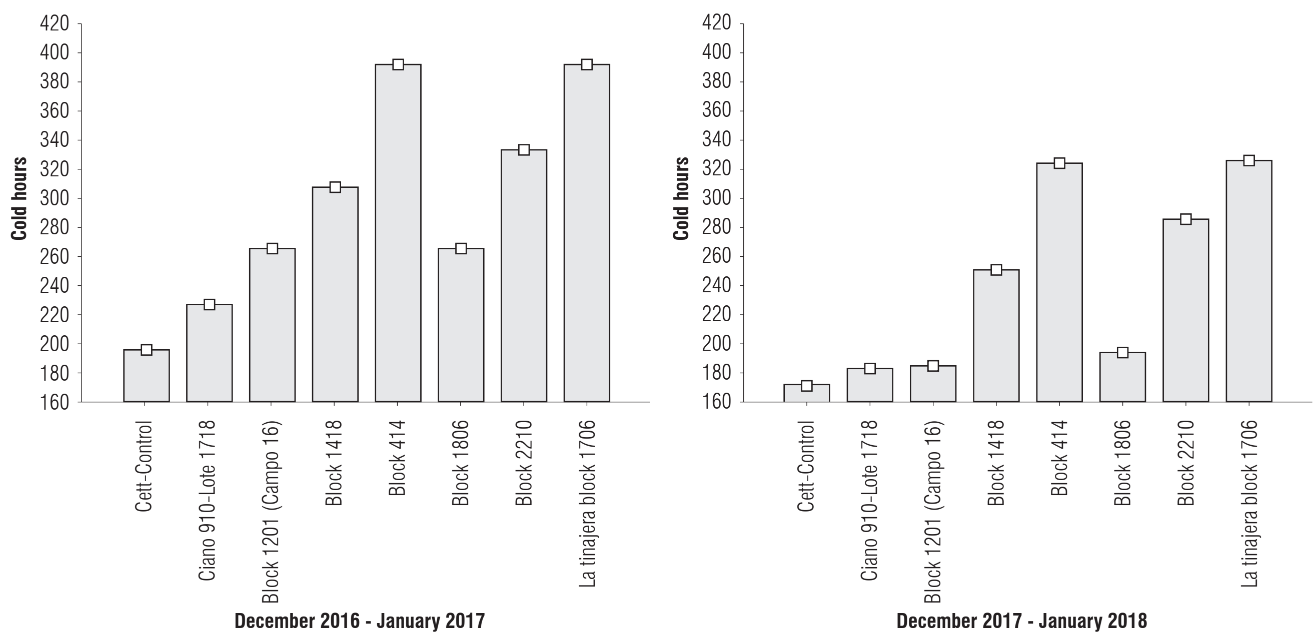

FIGURE 5. Cold hours during the December 2016 - January 2017 and December 2017 - January 2018 crop cycles. R^2: coefficient of determination without adjustment for the sites = 0.78, for cycles = 0.18 and their interaction = 0.04; CV: coefficient of variation = 29.72; Squares represent mean ± standard deviation.

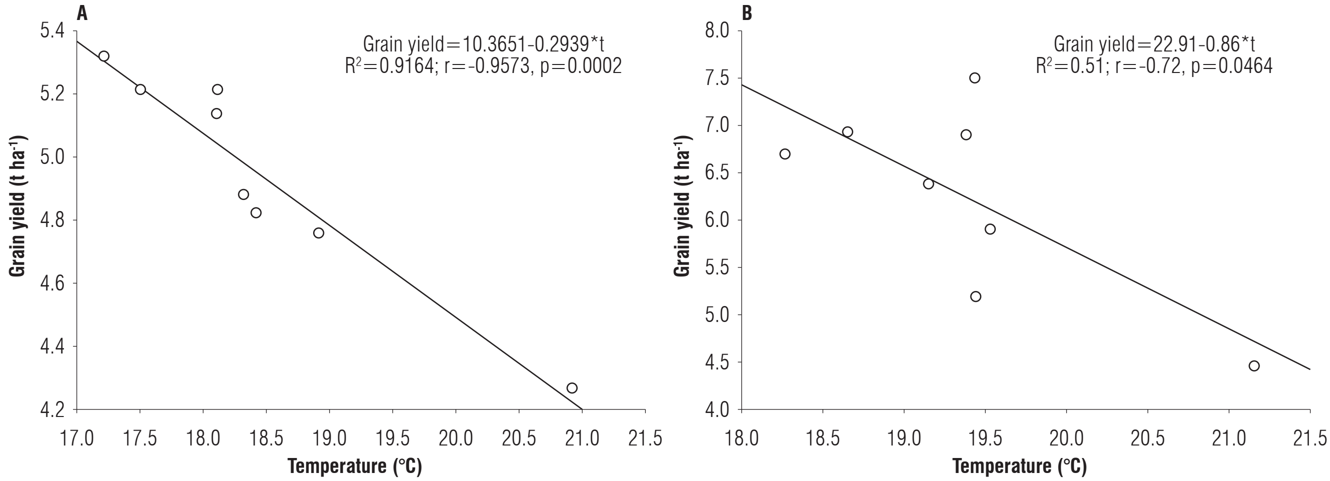

FIGURE 6. A. Effect of temperature on the grain yield during the 2016-2017, and B. 2017-2018 crop cycles. R^2: coefficient of determination without adjustment; r: regression coefficient; p: probability.

used to determine the theoretical temperature values that affected yield at 30%, between the temperature and grain yield. The first crop cycle had a theoretical temperature value of 23.7°C, whereas the second cycle had a value of 22.9°C, demonstrating a greater sensitivity of the wheat to temperature increases in the crop cycle 2017-18.

Warming stresses may cause a grain yield reduction through a reduction of spike number per m^2 (Shirdelmoghanloo *et al.,* 2016) and an increase of non-viable pollen production, which makes grain formation difficult (Siebers *et al.,* 2017). Currently, increased seed densities are used to overcome the temperature effect on tillering capacity reductions in a productive manner in agronomic practices (Lazzaro *et al.,* 2018), which greatly increase seeding costs (Chandra *et al.,* 2017).

Conclusions

A temperature variability of 1°C was observed between the 2016-17 and 2017-18 crop cycles.

The tiller number was significantly reduced from four to one tiller in both evaluated crop cycles.

The warming caused a significant decrease in the ABA content and an increase in the GA, which corroborated its contribution to the wheat tillering capacity reduction in Yaqui Valley.

Although the 2017-18 crop cycle had a higher grain yield, the temperature increase had a negative and significant effect on the grain yield at all evaluated sites and crop cycles in Yaqui Valley, Mexico.

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