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Reproductive and seminal characteristics of Pelibuey rams infected with *Mycobacterium avium* subsp. *paratuberculosis* in the subclinical stage

Velázquez-Morales, José V.[1,2]; Santillán-Flores, Marco A.[3]; Navarro-Maldonado, M. Carmen[4]; Salazar-Ortiz, Juan[5]; Ambríz-García, Demetrio A.[4]; Palacio-Núñez, Jorge[6]; Ponce-Covarrubias, José L.[7]; Cortez-Romero, Cesar[2,6*]

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ABSTRACT

Objective: To evaluate the reproductive and seminal characteristics of Pelibuey rams infected with *Mycobacterium avium* subsp. *paratuberculosis* in the subclinical phase.

Design/methodology/approach: In order to determine whether paratuberculosis (PTB) affects the reproductive variables and seminal quality in Pelibuey rams naturally infected with *Mycobacterium avium* subsp. *paratuberculosis* (MAP) in the subclinical phase, weekly evaluations were conducted, over a period of two months, of the variables live weight, scrotal circumference, ejaculation latency and seminal characteristics: volume, pH, masal motility and progressive individual motility, concentration, live spermatozoa and dead spermatozoa, in five rams infected by MAP and a further five uninfected rams, of average weight and age 53.58 kg (±3.26) and 2.91 years (±0.59), respectively.

Results: PTB was not found to affect the reproductive and seminal variables evaluated.

Limitations of the study/implications: It would have been convenient to carry out the evaluation of the seminal characteristics for a longer time, however, the sampling was stopped in order to ensure the animal welfare of the rams as far as possible, since they began to show signs of PTB.

Findings/Conclusions: The Pelibuey rams diagnosed with PTB in subclinical phase did not present any effect on the reproductive and seminal characteristics. Meanwhile, reproductive management can be carried out with PTB-infected Pelibuey lambs in a subclinical phase.

Keywords: Subclinical paratuberculosis, live weight, seminal evaluation, rams.

INTRODUCTION

Paratuberculosis (PTB) is a debilitating incurable disease that causes chronic granulomatous gastroenteritis with lymphangiectasia and lymphangitis. Animals infected with PTB, present chronic or intermittent diarrhea, hypoproteinemia, weight loss and eventual death; the

Image by david diaz at Pixabay

etiologic agent is *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and the disease mainly affects domestic and wild ruminants (Chiodini *et al.*, 1984). The main route of MAP transmission is fecal-oral (Clarke, 1997; Whittington & Windsor, 2009). Lesions caused by MAP can be presented 12 months prior to any evident clinical signs of PTB (Mcgregor *et al.*, 2015). Progression and outcome of the disease varies considerably according to the individual; clinical signs usually develop over the months or years following exposure, depending on the species (Whittington *et al.*, 2012). Likewise, it is considered that the clinical cases represent the tip of the iceberg, since it is estimated that for each clinical case detected there are at least another 25 potentially infected animals, with no apparent clinical signs (Whitlock & Buergelt, 1996). However, some factors have been described that cause stress in the ovine; such as malnutrition and parasitic, viral or bacterial infection, that can influence the transition from the subclinical to the clinical stage of PTB (Ayele *et al.*, 2001).

In Mexico, the rate of prevalence of PTB is between 5 and 30%, mainly in bovines, goats, ovines and fighting bulls (Guzmán-Ruiz *et al.*, 2016). With reference to these seroprevalence values, Stau *et al.* (2012) indicated that the most frequently used diagnostic test is the enzyme linked immunosorbent assay (ELISA), which has been used to detect antibodies for MAP since it is of low cost and the collection of samples and test procedures are easy, with little risk of contamination, and produce results faster than other diagnostic tests. A seroprevalence of 20% has been detected in bovine semen production units, raising the possibility of propagating the disease to a large number of females during artificial insemination (Abbas *et al.*, 2011). This could also occur in ovine, since the presence of MAP has been reported in infected rams (Eppleston & Whittington, 2001). In terms of the risk of transmission of MAP, Ayele *et al.* (2004) reported that the use of bovine stud bulls infected with PTB in subclinical state could present a risk for the dissemination of the mycobacteria via semen, since the presence of MAP has been reported in testicles, epididyme, seminal vesicles and semen. In the case of infected ovine, Eppleston & Whittington (2001) indicated the presence of MAP in the mesenteric lymph nodes, ileon and semen, and Velázquez-Morales *et al.* (2019) identified MAP in semen samples (42.9%) and in testicular tissue (42.8%) wearing the nested PCR technique, in rams Pelibuey with PTB in a clinical stage. In terms of the effects of PTB on semen, Khol *et al.* (2010) reported reduced motility and integrity of the membrane and morphology of the normal spermatozoid in an infected bovine during the transition from the subclinical to the clinical stage of the disease. However, no studies have shown whether PTB affects the seminal characteristics of naturally infected rams, which would represent a risk in the use of ovine semen in genetic improvement programs. Therefore, the objective of this study was to evaluate the live weight (LW), scrotal circumference (SC), ejaculation latency (EL) and seminal characteristics in rams naturally infected with MAP, in subclinical stage.

MATERIAL AND METHODS

Accommodation and animals

The experiment was conducted in the observation and isolation corrals of the ovine flock of the Córdoba Campus of the Colegio de Postgraduados (ColPos), a research and study unit with a background of prevalence of PTB. The rams were kept in different facilities in separate corrals in order to avoid possible infection, but under the same conditions of intensive management, which allowed the practice of protocols of control and prevention of naturally infected ovines. The experiment was designed under the criteria of the Mexican Official Norm (NOM-062-ZOO-1999) covering technical specifications for the production, care and use of laboratory animals (SAGARPA, 2001), in concordance with the regulations for the use and care of research animals (ColPos, 2019). The Campus is located at Córdoba-Veracruz federal highway (18° 51"20"' N; 96° 51"37"' W, and 720 meters above sea level). The climate is warm subhumid, with a mean temperature of 18 °C and mean annual precipitation of 1807.3 mm (García, 2004). Ten rams of the Pelibuey breed were used: five infected with PTB in subclinical stage and five non-infected, with mean weight and age of 53.58±3.26 kg and 2.91±0.59 years, respectively. Each ram was given 2.0 kg with alfalfa hay (18% PC) per day, with 200 g of concentrate (14% PC; 1.7 DS/kg) and water was provided freely.

Diagnosis of PTB

To diagnose PTB in the Pelibuey rams, the serological ELISA test was performed at the beginning and end of the experimental phase. This test presents a sensitivity of 79.31% and specificity of 82.25%, according to Martínez-Covarrubias *et al.* (2012). The test was conducted with blood samples taken from the jugular vein. The blood serum was recovered by centrifugation at 1000 x g for 10 min, and stored at −20 °C until subsequent processing in the CENID (Centro Nacional de Investigación Disciplinaria)-Microbiology laboratory of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP).

Measurement of reproductive variables and collection of ejaculate

During the reproductive season (october and november), eight measurements were taken weekly in the mornings. The parameters measured were live weight (LW), scrotal circumference (SC) and ejaculation latency (EL), and semen was collected. The measurements of LW and SC were conducted prior to initiating the semen collection. For measurement of LW, an electronic balance of capacity 250 kg ± 100 g (Braunker YP200S) was used; for SC, a flexible metallic scrotometer was used, following the methodology indicated by Benmoula *et al.* (2017). The EL measurement was conducted with each collection of semen, following that proposed by Swelum *et al.* (2017). Prior to beginning semen collection, the preputial hair was cut, and the prepuce was washed with antiseptic liquid soap and disinfectant (Dermocleen^{MR}). Seminal samples were then obtained from each individual using the artificial vagina method in accordance with that established by Williams *et al.* (2001).

Semen evaluation

Immediately after semen collection, the seminal quality was evaluated with macro and microscopic tests. The variables of the seminal evaluation were: volume (V, mL), masal motility (MM, classification from 0 to 5), individual progressive motility (IPM, %), spermatic concentration (C; value × 10^6 spermatozoids mL^{-1}), live spermatozoids

(LS, %) dead spermatozoids (DS, %) and pH (values from 0 to 14). Examination of the V and IPM and estimation of MM were conducted following the protocol of Benmoula *et al.* (2017). Determination of V was conducted in graduated collection tubes of 15 mL, at a temperature of 37 °C. The IPM was determined under a microscope (objective 400 x), with values expressed as percentages from 0 to 100. To determine MM, the semen was observed under a microscope (objective 10 x) (Carl ZEISS, Primo Star, CP11406, Microimaging GmbH 37081, Gottingen, Germany, Series-Nr: 3125001511), with values assigned from 0 (null motility) to 5 (vigorous motility). The percentage values of LS, DS and C were determined following the methodology of Ogundele *et al.* (2016); LS and DS were calculated from a smear stained with the eosin-nigrosin technique, in which the live and dead spermatozoids were quantified using bright-field microscopy (400 x). The value of C was determined using the hemocytometer method, visualizing the semen (objective 40 x) in a NeuBauer chamber (Marienfeld®). The pH was measured using paper pH test strips (Macherey-Nagel GgmbH and Co. KG) with a pH interval of 6.5 to 14 (El'Tohamy *et al.*, 2012).

Statistical analysis

The experimental design was a completely randomized model with repeated measures over time. The infected animal was used as a fixed effect and each animal nested in the treatment with PTB used as a random effect. For analysis of all variables, a Mixed procedure was used in the statistical package SAS® (SAS Institute 2006). Comparison of means was conducted with a Tukey test, employing a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Live weight, scrotal circumference and ejaculation latency

The presence of MAP in the rams infected with PTB in subclinical stage did not affect LW, SC and EL ($p>0.05$) over a period of two months (Table 1).

The results of this study indicated that PTB in subclinical stage had no effect on LW. Despite the fact that PTB is a disease characterized by causing weight loss, dehydration and profuse diarrhea in adult bovines, this diarrhea is not a constant in ovines (Verin *et al.*, 2016). Likewise, the presence of diarrhea was not reported in this study and no effect was found on LW. In contrast, Mcgregor *et al.* (2015) reported an effect on LW when they conducted a three-year longitudinal study in PTB infected Merino sheep of up to three years old, which could involve a weight loss of up to 5 kg. That study stated that

Table 1. Live weight, scrotal circumference and ejaculation latency in Pelibuey rams with and without PTB in subclinical stage, over a period of two months.

Animals	Variables		
	Live weight (LW, kg)	Scrotal circumference (SC, cm)	Ejaculation latency (EL, s)
With PTB	54.3±3.2	28.6±1.47	48.8±8.09
Without PTB	54.5±3.2	30.4±1.47	40.7±8.02

No differences were found between treatments (Tukey; $p>0.05$). Mean ± standard error.

the weight loss was the result of the effects of intestinal lesions caused by MAP, which generally occur at two years after exposure. It should be noted that, unlike in the males, the females more habitually present the effects of protein metabolism, catabolism and mobilization of fats, which can be particularly notable in ewes at the end of pregnancy or in early lactation when the demands of synthesis of energy and proteins are increased (Allen *et al.*, 1974). This supports that stated by Clarke (1997) and Jaimes *et al.* (2008), whom indicate that in small ruminants, PTB is manifested at between two and three years old, due to the diffuse hypertrophy caused by MAP in the mucous membrane of the jejunum and ileon; these lesions typical of MAP (granulomatous enteritis and thickening of the intestinal mucous membrane) cause poor absorption of nutrients with enteropathy, and thus generate a loss in corporal condition. However, it has not been determined whether these lesions present in subclinical stage reduce the intestinal function to a degree that is sufficient to produce a negative energetic balance (Kostoulas *et al.*, 2006). In relation to SC, no differences were found between treatments in this study; further, it was demonstrated that PTB did not generate a reduction in SC. To date, there is no literature that reports alterations in the SC of rams infected with MAP; however, Khol *et al.* (2010) took measurements of the epididyme over a period of one year in a bovine infected with MAP and reported a diameter decrease of 3 cm. Smith *et al.* (2010) indicated the likelihood that PTB acted to reduce fertility in dairy cows, due to the fact that the infection generates a negative balance of energy and protein, but not because of any direct effect of MAP at the reproductive level. This coincides with that found in the present study, since no modifications were found in the values produced by the weekly evaluations conducted, demonstrating that the variables LW, SC and EL presented no changes, at least during the sampling period.

Seminal quality

The quality of the ejaculate showed no variation among the rams with and without PTB infection ($p>0.05$; Table 2) over the two-month period of the test.

The repeated measurements of V and C did not present variation over time as a result of the effect of MAP ($p>0.05$). Again, no effect was found in the measurements of pH,

Table 2. Seminal characteristics in Pelibuey rams with and without PTB in subclinical stage, over a period of two months.

Variables	Rams	
	With PTB	Without PTB
Volume (V, mL)	0.9±0.09	0.9±0.09
pH	6.9±0.13	6.9±0.13
Masal motility (MM, 0-5)	3.8±0.21	4.2±0.20
Individual progressive motility (%)	82.7±2.83	85.5±2.76
Concentration (C, 10^6 spermatozoa mL^{-1})	2,420±425.4	2,634±425.4
Live spermatozoa (LS, %)	83.2±5.8	81.3±5.8
Dead spermatozoa (DS, %)	16.7±5.8	18.7±5.8

No differences were found between treatments (Tukey; $p>0.05$). Mean±standard error.

LS and DS (Table 2) taken over the two-month period of testing ($p>0.05$). The presence of MAP also had no influence on the values of the repeated measurements over time in the variables MM and IPM ($p>0.05$). Khol *et al.* (2010) reported the case of a bovine in which seminal evaluations were conducted over the period of one year, which showed variations in V and pH of between 4 and 12 mL and between 6 and 7.4, respectively; the MM decreased from 72 to 49%, and C never exceeded 2.8×10^6 per mL. For this reason, the semen was considered to be of poor quality, but these authors indicated that this low seminal quality was due mainly to malnutrition and the poor corporal condition of the animal and was not a consequence of infection with MAP. In addition, Caldeira *et al.* (2021) described that Bulls Nelore semen inoculated with MAP (10^3 to 10^8 CFU/mL) generates a decrease in sperm motility and vigor, which is possibly due to the adhesion of MAP with the intermediate part (tail) of the sperm, region containing a large amount of fibronectin in the plasma membrane.

Since the infection in the rams of this study was acquired naturally, it should be noted that the duration of the period of this infection is unknown. For this reason, there is uncertainty regarding the number of granulamatomas present in the intestine. These are lesions that take years to produce changes in the corporal condition and health of ruminants (de Silva *et al.*, 2018). Clinical signs are generally presented at between two and five years old in bovines and ovines (Verin *et al.*, 2016). Since the age of the rams in this study was less than three years, it is possible that this prevented the progression of these lesions, since PTB is a chronic disease that presents a prolonged preclinical phase (Subharat *et al.*, 2012). It is also important to mention that the two month duration of the study is a relatively short period, which precluded recording of the progression and effects in the rams with PTB in subclinical stage.

For their part, de Silva *et al.* (2018) indicated that a chronic disease such as PTB requires years to develop under natural conditions, and that an experiment lasting 12 months therefore represents a short period. For this reason, the duration of the present study (two months) could not demonstrate whether PTB in subclinical stage affects the rams in terms of productive and reproductive variables. Nevertheless, it is important to highlight the risk presented by the use of naturally infected stud rams, even in the absence of clinical signs and with no apparent change in seminal variables during their productive lives, as shown in the results of this study with Pelibuey rams. For their part, Eppleston & Whittington (2001) indicated that infected rams can carry MAP in the semen, and can thus transmit the infection horizontally. For this reason, any possibility of the propagation of PTB is of great concern due to the associated considerable economic losses and risks to public health, since it has been related to Crohn's disease in humans (Ghadiali *et al.*, 2004; Garcia & Shalloo, 2015). In this regard, Jaimes *et al.* (2008) indicate that control of the disease in domestic ruminants depends on the early detection and elimination of infected animals, although this is limited by the current lack of an adequate diagnostic. It is therefore necessary to implement strategies of vigilance, detection and control of MAP, despite the fact that rams infected with PTB in subclinical stage present reproductive capacity and are phsyiologically fit.

CONCLUSIONS

The Pelibuey rams diagnosed with PTB in subclinical stage did not present any effect on the variables LW, SC and EL attributable to the presence of the MAP. Equally, the seminal variables V, pH, MM, IPM, C, LS and DS presented no significant differences as a result of the infection with MAP.

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AGRO PRODUCTIVIDAD
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Effect of gypsum and potassium on corn yield and on the exchangeable bases of an acid soil in La Frailesca, Chiapas

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ABSTRACT

Objective: To evaluate the residual effect of gypsum in corn crops (2 years after its application in a previously limed soil), as well as the result of a new addition of gypsum combined with potassium in La Frailesca, Chiapas, Mexico.

Design/Methodology/Approach: We used a composite factorial design. The initial arrangement (2017) consisted of four levels of gypsum (0, 1.25, 2.5, and 5 t ha-1) and four levels of potassium (0, 60, 120, and 180 kg K_2O ha^{-1}). In 2019, the gypsum-treated plots were divided in half: the same amount of gypsum applied in 2017 was added to the first half and the other half was used to assess the residual effect of the initial treatment. The potassium doses were the same as the original. Corn grain yield, pH, exchangeable bases, and aluminum saturation percentage were measured at 0 to 7 and 7 to 14 cm below ground level.

Results: The greatest effect on yield was obtained with 2.5 t ha^{-1} of gypsum applied in 2017; no significant increases were recorded with higher gypsum doses. The exchangeable calcium content and pH level increased, while magnesium, potassium, and aluminum in the soil decreased.

Study Limitations/Implications: Suspected presence of Tar Spot Complex was diagnosed.

Findings/Conclusions: An excessive application of gypsum generates an imbalance in exchangeable potassium and magnesium in the soil; therefore, producers must exercise caution in the use of these products as part of their fertilization plan.

Keywords: Frailesca, acidity, gypsum, potassium, aluminum.

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INTRODUCTION

The Frailesca region is considered the granary of Chiapas, due to its high corn production. Its Regosols have low exchangeable calcium (Ca), magnesium (Mg), and potassium (K) contents. Among other factors, this phenomenon is caused by the erosion resulting from the burning of crop residues (that leaves the soil unprotected from the intense rainfall), as well as from the excessive use of ammonia fertilizers (that contribute to its acidification).

Image by Hans at Pixabay

Furthermore, the high exchangeable aluminum (Al) content in the soils limits the land productivity of approximately 35,000 ha^{-1} cultivated with corn, where yields ranging from 0.8 to 1.2 t ha^{-1} are obtained, affecting the income of around 14,000 producers.

Dolomitic lime could be applied to counteract the acidity of the soil, at a depth of 0 to 15 cm (Bossolani *et al.*, 2020). This product has local action, because of its low solubility (0.013 g L^{-1}) (Zapata, 2014). Its application dose in La Frailesca is calculated with the formula proposed by Yost (1991), modified, and adjusted by Camas *et al.* (2019).

The addition of Ca improves the fertility of acid soils; dolomitic lime and agricultural gypsum contribute Mg and S, respectively. Zoca and Penn (2017) pointed out that there is no exact scientific method to accurately and a priori estimate the gypsum that must be added to the soil.

The recommended dose of gypsum for the Frailesca is the result of the work carried out by Tasistro *et al.* (2015), who established a 1.25 t ha^{-1} of gypsum as an agronomically and economically recommended dose, during the first year of evaluation. These authors also reported that most of the soils in this area have low concentrations of exchangeable K and that K deficiency symptoms are common in corn crops. However, there is insufficient evidence about how this crop responds to the application of K; this response is attributed to the limitations imposed by soil acidity. The objective of this work was to evaluate the residual effect of agricultural gypsum and potassium on corn yield (2 years after their application), as well as the result of a second amendment application (particularly on exchangeable soil bases).

MATERIALS AND METHODS

Corn was used as an indicator crop for this experiment, which was carried out in the municipality of Villa Corzo, Chiapas (16.2025 N and −93.3382 W), an area characterized by hillocks, variable fertility, and scarce organic matter. The local soil belongs to the Dystrudepts group (Tasistro *et al.*, 2022). The trial followed up an experiment established in the spring-summer (SS) 2017 cycle to measure the response to the application of dolomitic lime, gypsum, and potassium. The applied lime dose was calculated using Yost's formula (1991) for an aluminum saturation of 47%, at a depth of 0 to 13 cm. The said dose was applied to the entire experiment.

The 2017 treatments consisted of a factorial arrangement of four levels of gypsum (0, 1.25, 2.5, and 5 t ha^{-1}) and four levels of potassium chloride (0, 60, 120, and 180 kg K_2O ha^{-1}) arranged in random blocks. The experiment consisted of three replications per treatment.

For the SS 2019 cycle, the experimental units with 1.25, 2.5, and 5 t ha^{-1} of gypsum were divided into two, with four 6-m long rows and 0.8 m between rows each one. The same dose of gypsum was applied to one half and the other half was used to evaluate the residual effect of the 2017 application. In the resulting 28 treatments, the potassium levels were the same as in the first application, while the controls did not receive doses of gypsum and potassium.

On July 6, 2019, the H-318 hybrid was manually established, depositing one seed every 19 cm; consequently, an approximate density of 65,000 seeds ha^{-1} was obtained. One day after sowing, 20% (*i. e.* 40 kg ha^{-1}) of the total scheduled N (200 kg ha^{-1}), 100% of the phosphorus (60 kg P_2O_5 ha^{-1}), and 100% of K (0, 60, 120, and 180 kg K_2O ha^{-1}) were applied. The treatment was applied in a line that ran 10 cm apart from the planting furrow. In the V5 and V10 phenological stages (Ritchie and Hanway, 1982), 40% of N (80 kg ha^{-1}) was applied in each one. The sources of the fertilizers used were urea, diammonium phosphate, and potassium chloride. The second application's gypsum was distributed over the soil 10 days after the emergence of the corn and was incorporated with a mattock at a 7.0 cm depth. This amendment contained calcium carbonate as impurities (32% PRNT).

The corn was grown under an efficient agronomic management, avoiding the presence of other factors that could interfere with those of the study. The response variable measured was corn grain yield, expressed on a 14% moisture basis. The harvest was carried out in the two central furrows of each plot, excluding 1 m at each end of the plot.

At the beginning and end of the SS 2019 cycle, composite soil samples were collected from each plot. Each sample consisted of 16 subsamples obtained from the two central furrows at two depths (0 to 7 and 7 to 14 cm), including the area where the fertilizer was applied alongside the furrow.

The chemical determinations carried out on the soil samples were pH in 1N KCl; exchangeable Ca, Mg, Na, and K with 1N ammonium acetate pH7 (Knudsen *et al.*, 1982), exchangeable acidity ($Al^{3+}+H^{+}$) in 1N KCl (Lin and Coleman, 1960). The effective cation exchange capacity (ECEC) and the Al saturation percentage were calculated based on the previous data. An analysis of variance (ANOVA) —using the test described by Shapiro-Wilk (α=0.05)— was performed to determine normality and Levene's test (α=0.05) was used to verify the homogeneity of variances.

All variables were analyzed using a randomized complete block factorial design. The factors were: depth (0 to 7 and 7 to 14 cm), number of gypsum applications (2017 and 2017+2019), and potassium dose (0, 60, 120, and 180 kg K_2O ha^{-1}). The pH values were transformed into the antilogarithm of the molar concentration of hydrogen ions. Meanwhile, the percentage data of the Al saturation were transformed by arcsin of the square root. The ANOVA was performed with the INFOSTAT V.20181 software and, when a significant difference was found, a comparison of means was made through Tukey's test (α=0.05).

RESULTS AND DISCUSSION

Effect of gypsum and potassium on corn grain yield

Corn grain yield was affected by gypsum, but not by potassium or gypsum×potassium interaction. Although the levels of exchangeable K in the soil increased according to K_2O increases, these levels did not influence the yield, because they did not exceed the critical level of 0.23 mEq/100 g (FAO, 2013).

The highest corn yield (6.2 t ha^{-1}) was obtained with the 2.5 t ha^{-1} of gypsum treatment applied in 2017; this result was like that achieved with the application of 2.5 t ha^{-1} (6.0 t ha^{-1}), divided into two doses of 1.25 t ha^{-1} of gypsum each (one applied in 2017 and another in 2019). Clearly, similar yields can be obtained with the application of either modality. Tasistro *et al.* (2022) indicated that, in agronomical and economical terms, the 1.25 t ha^{-1} dose of gypsum was the most adequate in the first year of application. However, our results suggest that, after two years, a new 1.25 t ha^{-1} dose has to be applied, given the residual loss of the amendment; another option is to apply 2.5 t ha^{-1} of gypsum in the first year to obtain a residual effect until the third year.

The highest gypsum doses applied —5 t ha^{-1} in 2017 or 10 t ha^{-1} (5 t ha^{-1} in 2017 and 5 t ha^{-1} in 2019)— led to a decreasing trend in corn grain yield (Figure 1).

At the beginning of the experiment (2017), prior to liming, the Al saturation percentage at 0 to 13 and 13 to 27 cm depths was 47% and 63%, respectively —which are considered toxic levels. This percentage substantially decreased, reaching less than 10% at the beginning of 2019; however, this decrease did not result in the increase in corn yields that same year. Tiecher (2018) pointed out that an increase in corn grain yield can be obtained, with a $<$10% Al saturation in the soil and when the application of rising doses of gypsum increases the Ca content. However, although both conditions were met in this study, no significant increase was observed. The second gypsum application (carried out in 2019) caused a decrease in the exchangeable K and Mg content of the soil and a change in their relationships with other cations, which could explain the lack of the expected response.

Effect of gypsum and potassium on soil pH

Figure 2 shows that, as the doses increased, the soil pH of the plots treated with gypsum showed an almost linear increase; this phenomenon was more evident in 2019. Our results suggest that a part of the 2017 amendment reached the deeper layers after 2 years, possibly because of the erosive effect. The soil had a sandy texture and scarce residue cover, as a result of the intense grazing to which it is subjected each year at the end of the harvest.

According to Bacca *et al.* (2011) gypsum (a neutral salt) has no effect on the pH. However, Tasistro *et al.* (2022) increased the amounts of this amendment in the same soil studied in this experiment (SS 2017 cycle) and reported pH variations in the order of 0.2 to 0.3 units. An *a posteriori* analysis of the gypsum used in this study determined the carbonate content as an impurity (32% PRNT), which would explain its influence on the soil pH at a depth of 0 to 7 cm and 7 to 14 cm (Zoca and Penn, 2017).

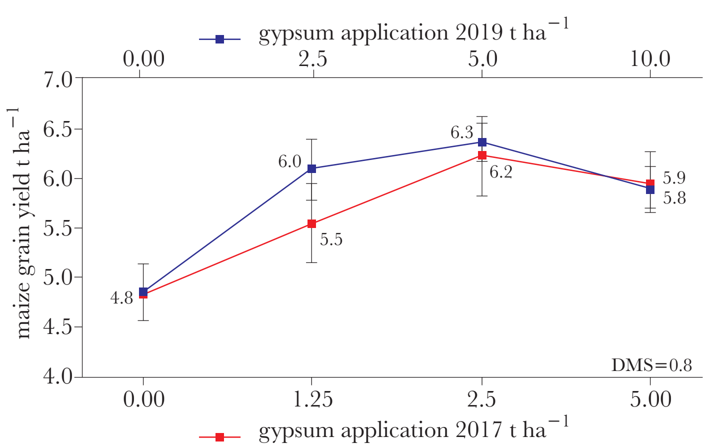

Figure 1. Effect of gypsum application in 2017 and 2019 on corn grain yield.

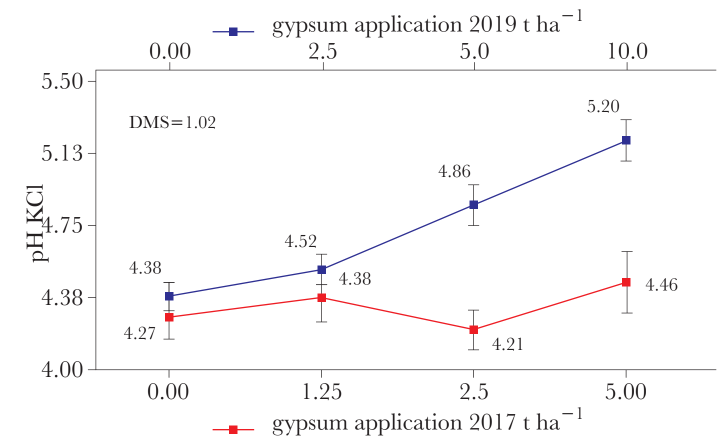

Figure 2. Effect of the gypsum application (2017 and 2019) on the pH at a depth of 0 to 7 and 7 to 14 cm.

Effect of gypsum and potassium on exchangeable calcium in the soil

The calcium extracted from the soil at a depth of 0 to 7 cm was higher as the gypsum dose increased (sum of the 2017 and 2019 applications) (Figure 3); however, the Ca content was lower at a depth of 7 to 14 cm. According to Caires (2004), the greater ionic radius of Ca (regarding Mg and K) and its lower mobility in the soil profile could cause this phenomenon.

Authors like Crusciol *et al.* (2016) observed that, 3 months after the application of gypsum, the Ca content in the tillable layer (0 to 10 cm) of the soil had increased; however, this element descended towards the 10 to 20 cm layer after a year. Therefore, the Ca in this study is likewise expected to reach deeper layers over time. If the objective of the practice is to increase the Ca concentration in the soil solution, gypsum is the most efficient amendment, since it can induce higher corn yields (Shainberg *et al.*, 1989). However, although the Ca contents increased in our study site during the SS 2019 cycle, no significant increases in corn production were observed, which suggests the existence of other nutritional problems (Figure 4).

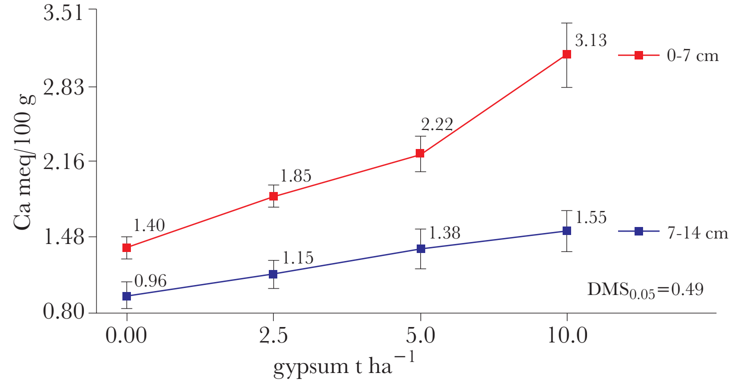

Figure 3. Joint effect of two gypsum applications (2017+2019) on exchangeable Ca, at 0 to 7 and 7 to 14 cm depths.

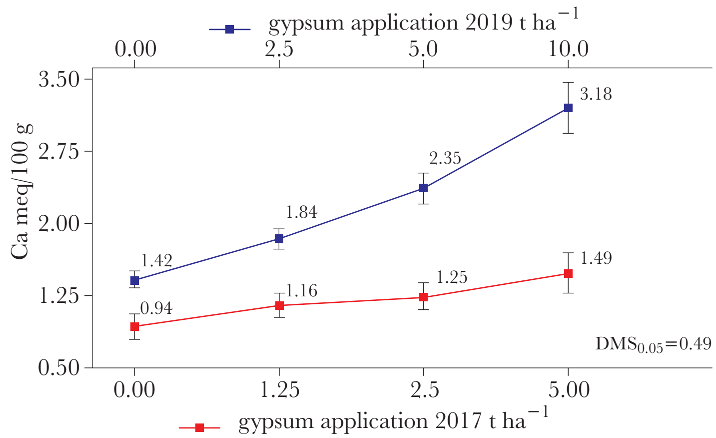

Figure 4. Effect of gypsum application (2017 and 2019) on the exchangeable Ca in the soil.

Effect of gypsum and potassium on the exchangeable magnesium in the soil

As a consequence of the generalized application of 1.9 t ha^{-1} of dolomitic lime (2017), the levels of exchangeable Mg in the treatment without gypsum increased; however, as the doses of gypsum applied jointly in 2017 and 2019 increased, a decreasing trend was recorded (Figure 5). In 2017, the extractable magnesium in the 0 to 7 cm layer was above the 0.3 cmolc kg^{-1} critical level considered by Kopittke and Menzies (2007); meanwhile, it was lower in the 7 to 14 cm layer, possibly as a result of the incorporation of lime at a depth of just 10 cm.

The second application of gypsum carried out in 2019 caused a decrease in exchangeable Mg at a depth of 0 to 7 cm. However, at a depth of 7 to 14 cm, no significant changes in this element were recorded. As in other abovementioned cases, we assume that gypsum, despite its high solubility, did not reach the deepest layers of the soil. Zoca and Penn (2017) argue that Mg and K leaching largely depends on the amount of gypsum applied, as well as on the texture and mineralogy of the soil. The results suggest that adding magnesium fertilizer would compensate for its decrease.

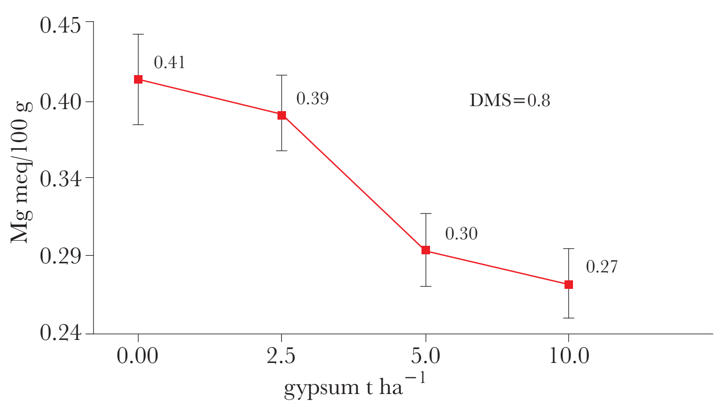

Figure 5. Joint effect of two gypsum applications (2017+2019) and two depths (0 to 7 and 7 to 14 cm) in the exchangeable magnesium of the soil.

Effect of gypsum on the exchangeable potassium in the soil

As the joint amounts of gypsum doses applied in 2017 and 2019 increased, the exchangeable K decreased (Figure 6), below the 0.23 cmol/100 g critical level in the soil (FAO, 2013).

Gonzalez *et al.* (2005) and Sparks (2003) report similar results in sandy soils like the findings of the study site. The application of high amounts of gypsum in their study displaced potassium to deeper layers. Therefore, the recommendation regarding the amount of gypsum to be used to correct the exchangeable acidity must be followed with care, taking into consideration the additional K contributions required to compensate for the decrease caused by the use of gypsum.

Effect of gypsum and potassium on the exchangeable aluminum in the soil and aluminum saturation

K fertilization by itself had a significant effect on the Al saturation percentage. With the widespread application of lime in 2017 and gypsum in 2017 and 2019, AI saturation dropped to 8.1%, while, in the treatment with 60 kg K_2O, it decreased to 4.3%. A similar behavior was recorded with higher amounts (Figure 7).

This indicates that, when lime and gypsum are used to correct the Al excess, part of the temporarily unavailable K is released; by adding K_2O, K can actively participate in the neutralization of the charges of the exchange capacity in the sites previously occupied by Al and the gypsum and the lime are released to the soil solution by the application of the amendment. The exchangeable Al and the Al saturation percentage were affected by the application of gypsum (2017+2019) at both soil depths (Figure 7). At the 0 to 7 cm depth with both applications, the exchangeable Al was below the critical level (2 meq/100 g) (Casierra and Aguilar, 2007) and the Al saturation percentage was less than 20% (Tasistro, 2012), which suggests that the residual effect of the dolomitic lime and gypsum applied in 2017 persists.

In the 7 to 14 cm depth, significant changes were observed in exchangeable Al (Figure 8) and in the saturation percentage. Two years after applying the amendments, the

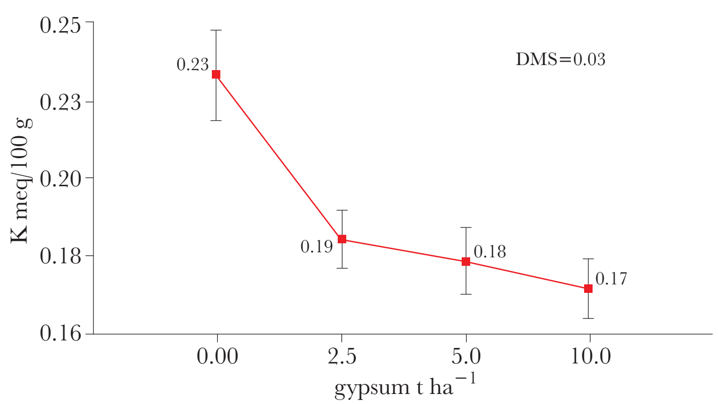

Figure 6. Joint effect of two gypsum applications (2017+2019) and two depths (0 to 7 and 7 to 14 cm) on the exchangeable K of the soil.

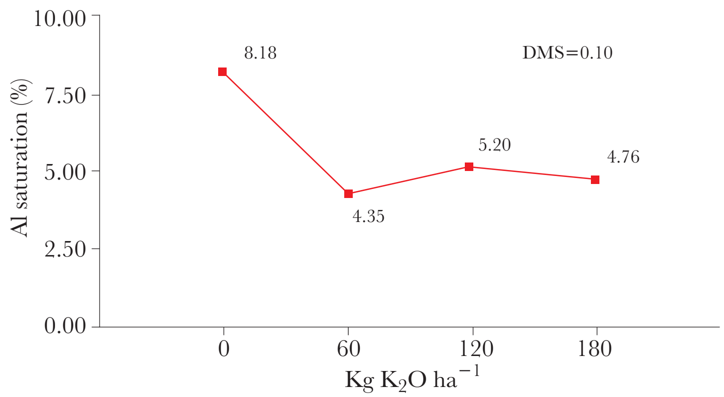

Figure 7. Effect of K_2O on the aluminum saturation percentage at a joint depth (0 to 7 and 7 to 14 cm).

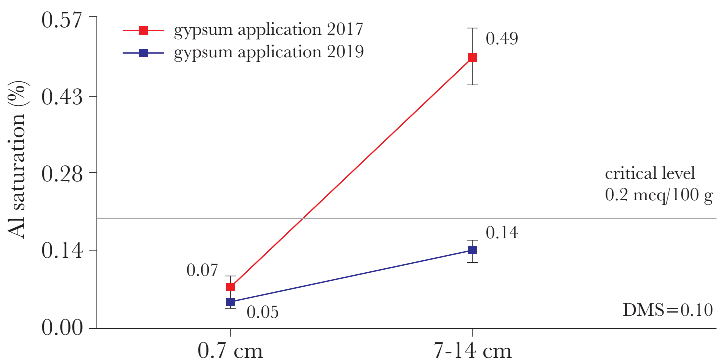

Figure 8. Effect of two gypsum applications (2017 and 2019) on interchangeable aluminum at two depths (0 to 7 and 7 to 14 cm).

exchangeable Al was above the critical level, which suggests that the gypsum may have moved downwards and reached deeper layers.

At that same depth, a second application of gypsum decreased the exchangeable Al by 0.35 meq, down to 0.14 meq and, on average, the Al saturation percentage decreased from 28% to 7%. In our study, the widespread application of dolomitic lime in 2017 (treatment 0 gypsum) kept Al saturation below the critical level, even after 2.5 years. The gypsum applications in 2017 and 2019 further decreased Al saturation; the effect was greater for the 2019 applications. This behavior may explain the increase in yield, both with 2.5 t ha^{-1} of gypsum in 2017 and the same amount but divided in two sets (1.25 t ha^{-1} each) in 2017 and 2019 (Figure 9).

Casierra and Aguilar (2007) suggest that the effect of Al on plant development depends on the tolerance of the species and on the physical and chemical properties of the soil. According to Ryan *et al.* (2011) the aluminum absorption mechanisms are not precisely known.

CONCLUSIONS

The application of gypsum resulted in increases in yield both with the treatment of 2.5 t ha^{-1} in 2017 (year 1) —which had a residual effect in the third year (2019)— and with the use of two similar applications of 1.25 t ha^{-1} of gypsum in 2017 and in 2019.

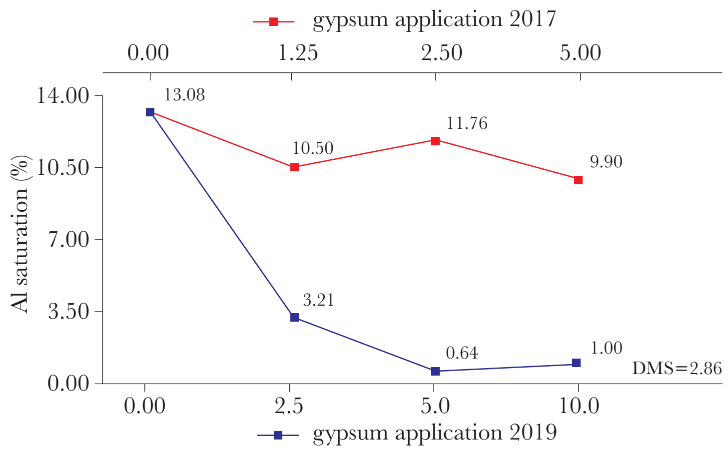

Figure 9. Effect of two gypsum applications (2017 and 2019) on the aluminum saturation percentage.

The application of higher gypsum doses in both years did not lead to an increase in corn yield; however, it increased the exchangeable calcium content and pH in the soil and decreased exchangeable magnesium, potassium, and aluminum.

The soil pH increased because of applying gypsum at a depth of 0 to 7 cm, as a result of the gypsum impurities (lime).

As a conclusion, the recommendations regarding the use of gypsum as an amendment should be taken with care since an excessive application generates a decompensation in the exchangeable K and Mg of the soil. Therefore, their incorporation into the fertilization plan should be carefully considered.

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AGRO PRODUCTIVIDAD

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Geopedological transects in karst landscapes in Campeche, Mexico

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ABSTRACT

Objective: To determine the spatial variability of landforms and their relationship with the soil geography of the state of Campeche, Mexico.

Design/Methodology/Approach: Two transects were carried out under the geopedological approach, using soil mapping and geomorphology material at landscape level. Geomatic techniques were used for the correction process, mapping the landforms at scale of 1:100,000. Soil profiles were developed westwards, giving priority to the diversity of landforms, resulting in geopedological transects.

Results: Plain landscapes (*e.g.*, P and Lf) feature hydromorphic processes, their soil is deep and rich in organic sediments, and the soil units were classified within the Histosols and Gleysols groups. The transitional EBDe landscape does not have an apparent dissection, presents relatively convex landforms (with a slight slope), and has moderately deep and well-developed soils —classified within the Cambisols group. Finally, the EBPD landscape presents higher elevation and dissection; its soil is mainly shallow with scarce or null pedon development and is related to convex landforms, while the soil units belong to the Leptosols group. For the elevated plains landforms, a relationship with the Luvisols group was determined.

Study Limitations/Implications: Understanding the geomorphology-soil relationships of a given region provides the basis for establishing soil distribution models —which will facilitate soil mapping and territorial planning.

Findings/Conclusions: Campeche's reliefs have a great complexity at landform level. Developing and updating the cartography of the land will help to improve the planning of productive and conservation projects.

Keywords: Geomorphology, plains, toposequences, karstic soils, Yucatan Peninsula.

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INTRODUCTION

Geomorphology is defined as the study of the various landforms of the earth's surface —originated by exogenous and endogenous processes— that constantly form and sculpt the land relief (García and Lugo, 2003). The Yucatan Peninsula is a relatively flat platform that consists of an earth system or morphogenetic environment and geomorphological landscapes (Lugo, 2011) and is typical of the states of Yucatán, Campeche, and Quintana Roo in eastern Mexico (Zavala-Cruz *et al.*, 2016; Fragroso-Servón *et al.*, 2019). Existing

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information points to landscape regionalization at the recognition and median scales, from which the importance for soil survey of the study of relief patterns can be inferred (Bautista-Zúñiga *et al.*, 2005b). According to Porta *et al.* (2003), pedology is the science that studies the soil. Therefore, geopedology sets forth geomorphological and pedological criteria to establish relationships between relief and soils (Zink, 2012; Zavala-Cruz *et al.*, 2016).

The geomorphological evaluation is a useful tool to provide information about the cartographic limits of the geoforms, as well as to determine the pedological characteristics that link them with the different forms of relief (Zinck, 2012; Zavala-Cruz *et al.*, 2016). Porta *et al.* (2003), Krasilnikov *et al.* (2011), and Zink (2012) point out that the relief determines the elevations within a territory and interacts with other landscape-forming factors (*e.g.*, climate and biota) to generate an effect on soil variability.

Nowadays, more emphasis has been given to the study of the relief-soil relationship, as a result of the interest in the implementation of plans with different approaches (Zavala-Cruz *et al.*, 2014; 2016; Aguilar-Rodríguez *et al.*, 2017; Palma-López *et al.*, 2017). Furthermore, the understanding of geomorphological aspects provides a better vision for the management of natural resources and is the basis for small- and medium-scale regionalization (Porta *et al.*, 2003; Zink, 2012; Zavala-Cruz *et al.*, 2016). The objective of this study was to describe the spatial variability of the relief at the landform level and its relationship with soils using geopedological regionalization in transects.

MATERIALS AND METHODS

Study area

The state of Campeche is located in the west and southwest of the Yucatan Peninsula, Mexico. It is located between the parallels 17° 40' 30" and 20° 50' 30" (N) and 89° 10' 30" and 92° 30' 00" (W). It has three climates which are distributed in different regions of the state: A(m), warm humid with rainfall in summer; A(w), warm sub-humid with rainfall in summer; and B(s), which refers to a dry steppe climate (García-Cruz, 2004). The precipitation captured in the different basins of the state is quickly evacuated by the prompt infiltration of the limestone rock; the dissolution of the rock creates underground rivers that flow into the sea through *petenes* (Bautista-Zúñiga *et al.*, 2005a). The geology of most of the territory is made up mainly of sedimentary rocks (limestone, marl, and gypsum) and, to a lesser extent, by sandstone and shales, as well as alluvial and marsh sediments (SGM, 2005; Zavala-Cruz *et al.*, 2016). The relief was classified under the approach of Zink (2012), arranged by morphogenetic environments —whose origin lay in endogenous and exogenous geodynamic processes—, controlling the modeling of geomorphological landscapes (which are characterized by their physiographic expression). Their differentiation was carried out with the following morphometric information: shape, slope, altitude, geomorphological or geodynamic process, and type and age of the rock (Ortiz-Pérez *et al.*, 2005; Zavala-Cruz *et al.*, 2016).

Geopedological regionalization of the relief

The present study was carried out through the interpretation and analysis of the geomorphological map at the landforms (LF) level, with a scale of 1:100,000. The map was developed with the Geographic Information System (GIS) and the ArcView 9.3 software, at the Laboratorio de Geomática del Colegio de Postgraduados, Campus Tabasco. The maps generated are based on regionalization studies at the earth system level, which refers to the morphogenetic environment and geomorphological landscapes described by Bautista-Zúñiga *et al.* (2005a) and Ortiz-Pérez *et al.* (2005). The limits of the said landscapes were perfected through the photo-interpretation and analysis of a Digital Elevation Model (DEM) (INEGI, 2011) and SPOT-type satellite images, generating a relief/modeling and LF repetitive pattern (Zink, 2012). Geomorphological processes and rock types were also identified to differentiate detrital, karstic, and marshy landscapes (SGM, 2005; Ortiz-Pérez *et al.*, 2005), among others.

First, we differentiated the LF-level reliefs for each geomorphological landscape; subsequently, we analyzed the distribution arrangement of the LFs, through two transects that were in an eastward direction (Figure 1): the AA1 and the BB1 transects.

Information about the soils of each geomorphological landscape was collected by describing soil profiles in the field, located according to the LF spatial distribution (Cuanalo, 1990; USDA, 2017). Each profile was described in terms of horizon, color, texture, consistency, structure, transition, presence of nodules, cutanes, waterlogging, and permeability, among others. Additionally, the description of the physical environment included the following elements: relief, slope, elevation, surface drainage, parent material, vegetation, and/or land use, as well as the dominant formation process. Subsequently, the soils were physically and chemically analyzed according to NOM-021-RECNAT-2000 (DOF, 2002), which establishes the regulations that oversee the analysis methodologies used for soil classification. In this way, through the world reference soil resource base of IUSS and WRB Working Group (2014), the soil mapping and classification was carried out.

RESULTS AND DISCUSSION

Geomorphological landscapes and landforms

Eight out of 23 differentiated geomorphological landscapes (Figure 1) were analyzed and characterized. They are located within the following systems or morphogenetic environments: SFP (Fluvial-Marshes System) and the SKT (Karst-Tectonic System). Figure 1 shows the list of geomorphological landscapes, as well as the spatial distribution of each unit, along with the location of the transects. The AA1 transect includes the P, Fp, and EBDe landscapes, up to the EBPD landscape —*i.e.*, it covers both morphogenetic or earth systems. Meanwhile, the BB1 transect covers the PRA, EBPD, and ADPC landscapes, up to the denudation highlands (DH), located at >200 m.a.s.l. Unlike AA1, this transect only represents the geomorphology-soil relationship of the SKT.

The LFs of the SFP morphogenetic environment, as well as of the SKT, have a depositional nature, with geomorphological landscapes of plains. For relief/modelling, two types were found in both environments: depression and floodplains. Being depositional, they are classified as unconsolidated materials (Zink, 2012); consequently, the facie in both environments is marshy and alluvial. Table 1 shows the morphometric information of the geomorphological landscapes and their respective LFs.

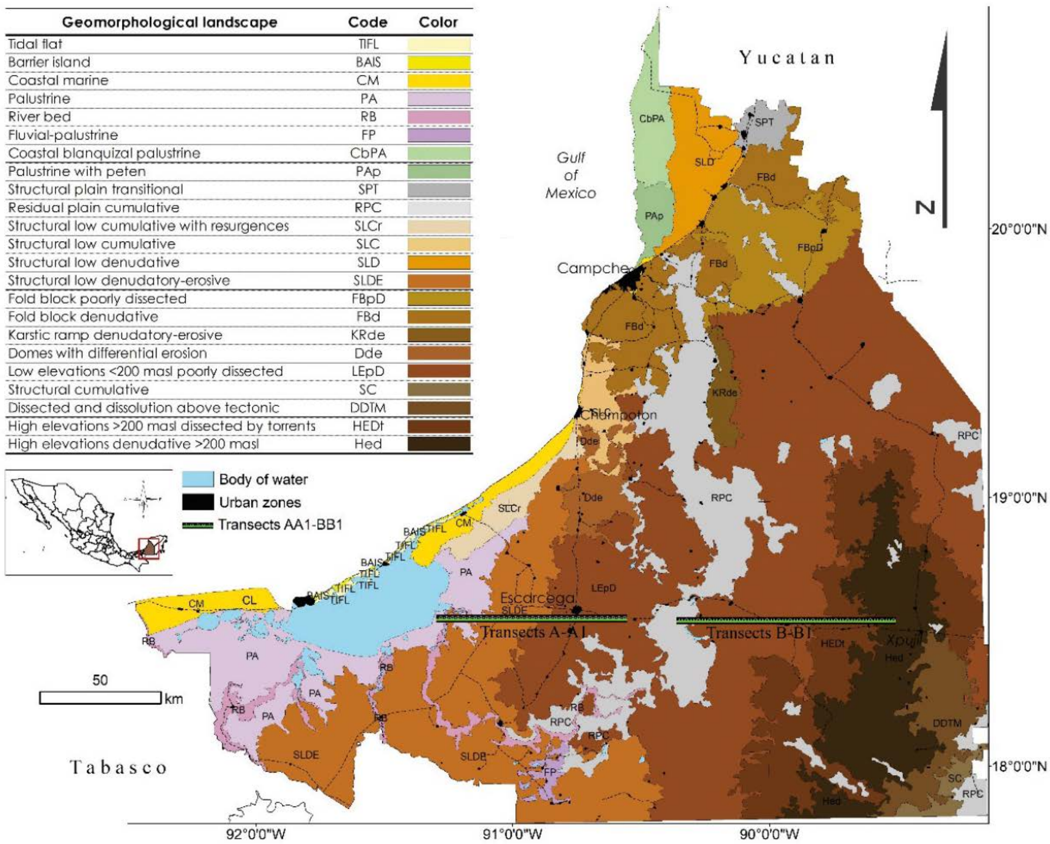

Figure 1. Map of geomorphological landscapes of the state of Campeche and geographical location of geopedological transects. Adapted from Lugo (2011), Bautista-Zúñiga *et al.* (2005b), and Ortiz-Perez *et al.* (2005).

Morphogenetic environment: SFP (Fluvial-Marshes System) and SKT (Karst-Tectonic System). **Geomorphological landscape**: P (marsh), Lf (river bed), EBDe (denudation-erosive low structural), EBPD (scantly dissected low elevations, <200 m.a.s.l.), PRA (cumulative residual plain), ADPT (highlands dissected by torrents, >200 m.a.s.l.), AD (denudation highlands, >200 m.a.s.l.). *Landforms*: Pbil (low lagoon floodplain), Llab (low floodplain), Llai (floodplain), T (terrace), Tc (karst terrace), Piac (wide cultivated inland plains), Piav (wide inland plains with vegetation), Ccxlo (slightly-undulating convex ridges), Ccxmo (moderately-undulating convex ridges), Ccxfo (strongly-undulating convex ridges), Prbdc (cultivated well-drained residual plain), Vt (torrent valley), Piacclo (broad inland plains and slightly-undulating convex ridges), Ccxlmo (slightly- to moderately-undulating convex ridges), Ccxmfo (moderately- to strongly-undulating convex ridges), Apbdv (highlands with well-drained plains with vegetation), and Alali (highlands with slightly-sloped isolated hills). **Geomorphological process**: A (accumulation), D (denudation), I (weathering), E (erosion), and K (karstification). **Rock type and age**: pa (marsh), al (alluvial), Ar (sandstone), Lu (shale), Cz (limestone), Mg (marl), Y (gypsum), Oho (Holocene - Quaternary), Opl (Pleistocene - Quaternary), Te (Eocene - Tertiary), and Tpa (Paleocene - Tertiary).

Table 1. Morphometric information of the geomorphological landscapes and landforms (LFs), in Campeche, Mexico.

Morphogenetic region	Geomorphologic landscape	TF	Altitude (masl)	Slope (%)	Geomorphologic process	Stone and age
SFP	P	Pbil	2-9	<1	C	pa, Qho
		Llab	9-10	<2	C	pa, Qho
	RB	Llai	10-11	<1	C	al, Qho
SKT	SLDE	T	11-40	1-6	W, E, C	Sn-Sh, Te-Qpl
		Tc	30-90	1-6	K, C	Ls-Lm, Te
		Piac	30-70	<2	C, K	Ls-Lm, Te
		Ccxlo	30-120	1-3	K, E, C	Ls-Lm, Te
	LEpD	Piac	30-140	<2	C, K	Ls-Lm, Ct, Te, Tpa
		Piav	35-140	<2	K, C	Ls-Lm, Ct, Te, Tpa
		Ccxlo	35-190	3-6	K, E, C	Ls-Lm, Ct, Te, Tpa
		Ccxmo	35-190	6-10	E, K, C	Ls-Lm, Ct, Te, Tpa
		Ccxfo	30-190	>25	E, K, C	Ls-Lm, Ct, Te, Tpa
	RCP	Prbdc	10-60	<1	C	al, Qho
	HEDt >200 masl	Vt	150-240	<3	C	Ls-Ct, Tpa
		Piacclo	200-250	1-6	C, K	Ls-Ct, Tpa
		Ccxlmo	200-250	3-10	K, E	Ls-Ct, Tpa
		Ccxmfo	200-250	10-25	E, K	Ls-Ct, Tpa
	HEd >200 masl	Apbdv	200-260	<2	C, K, D	Ls-Ct, Tpa
		Alali	200-260	3-6	K, E, D	Ls-Ct, Tpa

Each of the transects has an eastward altitudinal ascent. Baptist *et al.* (2005a), Ortíz-Pérez *et al.* (2005), SGM (2005), and Zavala-Cruz *et al.* (2016) point out that these landscapes —which correspond to SKT— have developed within a morphogenetic environment (earth system), where the dissolution of limestone rock and marls from the Tertiary Oligocene-Miocene prevails. Meanwhile, in the P and Lf plains, which correspond to the SFP morphogenetic environment, the parent material corresponds to unconsolidated sediments from the Quaternary - Holocene, mostly transported by the San Pedro and San Pablo River.

AA1 geopedological transect

Figure 2 and Table 2 shows the relationship between the relief and the soil of the AA1 transect, indicating that the SFP environment with depositional character has deep soils, with slight alkalinity, rich in organic materials. The said soils have poor to very poor internal drainage —which give rise to hydromorphic processes— and are classified as Histosols and Gleysols. They are related to concave LFs.

Soils in the SKT environment are mostly shallow, have high $CaCO_3$ concentrations and scarce or no soil development. They have been classified within the Leptosols group and were mainly related to convex LFs. To a lesser extent, deep, developed, and well-

Table 2. Soil units according to the landform in the AA1 transect.

Geomorphologic landscape	Terrain forms	Soil unit
PA	Low plain of lagoon flooding (Pbil)	Salic Histosols (Eutric) HS-sa.eu
	Low abyssal plain (Llab)	Molic Gleysols (Eutric, Clayic) GL-mo.eu.ce
RB	Flood abyssal plain (Llai)	Molic Gleysols (Eutric, Clayic) GL-mo.eu.ce
SLDE	Terrace (T)	Gleyic Cambisols (Humic, Clayic) CM-gl.hu,ce
	Karstic terrace (Tc)	Rendzic Leptosols LP-rz
LEpD	Wide cultivated interior plains (Piac)	Haplic Luvisols (Humic, Hipereutric) LV-ha-hu.he
	Wide interior plains with vegetation (Piav)	Leptic Luvisols (Hiperutric, Clayic) LV-lp.he.eu
	Slightly undulating convex crests (Ccxlo)	Hiperesqueletic Leptosols LP-hk.
	Moderately undulating convex tops (Ccxmo)	Hiperesqueletic Leptosols LP-hk.
	Strongly undulating convex crests (Ccxfo)	Hiperesqueletic Leptosols LP-hk.

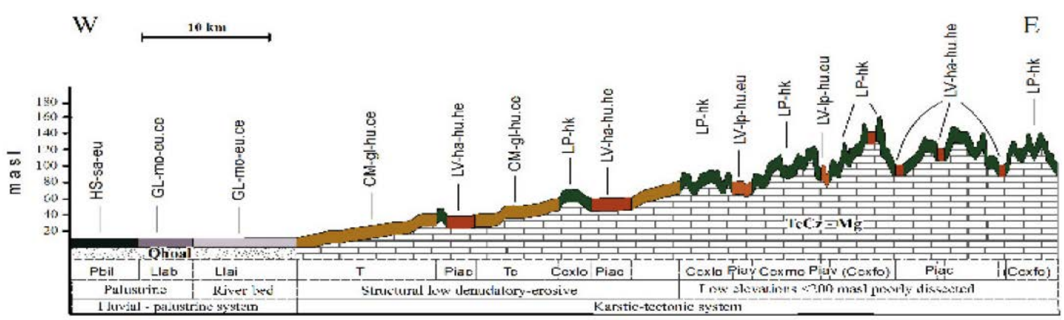

Figure 2. Geopedological toposequence of transect AA1. **Soil units**: HS-sa.eu Salic Histosols (Eutric), GL-mo-eu.ce Molic Gleysols (Eutric, Clayic), CM-gl-hu.ce Gleyic Cambisols (Humic, Clayic), LV-ha-hu.he Haplic Luvisols (Humic, Hipereutric), LP-hk Hiperesqueletic Leptosols, LV-lp-hu.eu Leptic Luvisols (Hiperutric, Clayic); **Terrain forms**: Pbil Low plain of lagoon flooding, Llab Low abyssal plain, Llai Flood abyssal plain, T Terrace, Tc Karstic terrace, Piac Wide cultivated interior plains, Ccxlo Slightly undulating convex crests, Piav Wide interior plains with vegetation, Ccxmo Moderately undulating convex crests, Ccxfo Strongly undulating convex crests.

drained soils have been recorded; they have been classified within the Luvisols group and are related to flat LFs. Unlike the above examples, the Terrace LFT —which is located in the transition area with the SFP— has a convex-concave to flat shape, with an extensive spatial arrangement, and is related to a deep soil with imperfect drainage. It has been classified within the Cambisols group. Similarly, the Tc with thin soil has been classified as

LP-rz. Specifically, the Ccxlo, Ccxmo, and the Ccxf LFs are directly related to the LP-hk soil unit, while the LV-ha-hu.he and LV-lp-hu.eu units —which are mainly located within the EBPD landscape, with some isolated remnants in the EBDe landscape— are connected with the Piac and Piav LFs, respectively.

BB1 geopedological transect

Figure 3 and Table 3 show the geomorphology-soil relationship of the BB1 transect for the SKT environment with dissolution and karstification processes. It is subdivided into four representative geomorphological landscapes: PRA, EBPD, ADPT >200 m.a.s.l., and AD >200 m.a.s.l. These landscapes have similar characteristics in terms of the repetition of LFs: the EBPD landscape has Piac and Piav, as well as Ccxlo, Ccxmo, and Ccxfo; the ADPT landscape includes Ccxlmo, Ccxmfo, and Vt; and AD >200 m.a.s.l. has Apbdv and Alali. This last landscape has the highest elevation in the study and extends into the state of Campeche (Bautista-Zúñiga *et al.*, 2005b).

The PRA landscape does not have isolated peaks or remains; the soil variability in this low-altitude landscape is residual and cumulative. Deep, developed, and well-drained soils predominate in these plains; they belong to the Nitisols group. At the same time, it is related to other groups of lower position that have not been included in the transect. The

Table 3. Soil units according to the shape of the terrain in the BB1 transect.

Geomorphologic landscape	Terrain forms	Soil unit
RPC	Well-drained and cultivated residual plain (Prbdc)	Molic Nitisols (Eutric, Rodic) NT-mo.eu.ro
LEpD	Wide cultivated interior plains (Piac)	Nitic Luvisols (Ferric, Hipereutric) LV-ni-fr.he
	Wide interior plains with vegetation (Piav)	Haplic Luvisols (Hipereutric, Esqueletic) LV-ha-he.sk
	Slightly undulating convex crests (Ccxlo)	Hiperesqueletic Leptosols (Rendzic) LP-hk-rz
	Moderately undulating convex tops (Ccxmo)	Hiperesqueletic Leptosols (Calcaric, Humic) LP-hk-ca.hu
	Strongly undulating convex crests (Ccxfo)	Hiperesqueletic Leptosols (Molic, Humic) LP-hk-mo.hu
HEDt >200 masl	Wide interior plains and slightly undulating convex crests (Piacclo)	Rendzic Leptosols (Humic) LP-rz-hu
	Stream valley (Vt)	Molic Gleysols (Calcaric, Humic, Clayic) GL-mo-ca.hu.ce
	Slightly to moderately wavy convex crests (Ccxlmo)	Litic Leptosols (Calcaric) LP-li-ca
	Moderately convex to strongly wavy crests (Ccxmfo)	Litic Leptosols (Calcaric) LP-li-ca
HEd >200 masl	High well-drained plains with vegetation (Apbdv)	Rendzic Phaeozems (Clayic) PH-rz-ce
	High with slightly sloping isolated hills (Alali)	Rendzic Leptosols (Clayic) LP-rz-ce

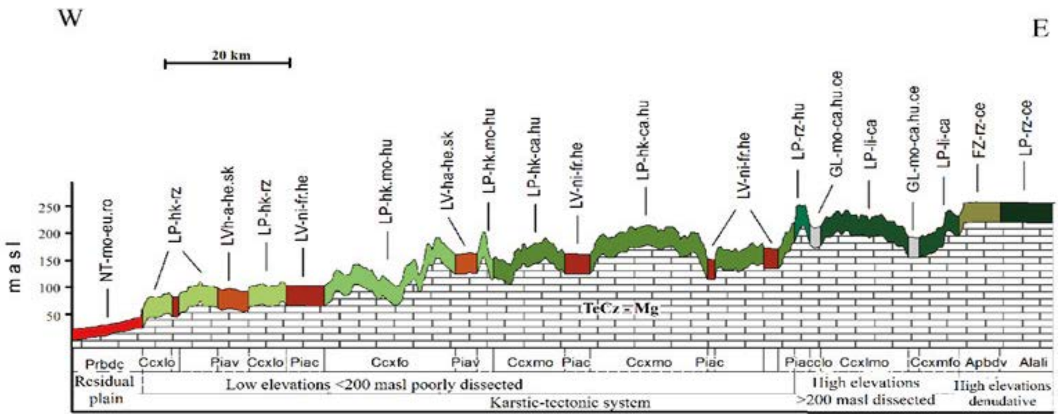

Figure 3. Geopedological toposequence of transect BB1. **Soil units**: NT-mo.eu.ro Molic Nitisols (Eutric, Rodic), LP-hk-rz Hiperesqueletic Leptosols (Rendzic), LV-ha-he.sk Haplic Luvisols (Hipereutric, Esqueletic), LV-ni-fr.he Nitic Luvisols (Ferric, Hipereutric), LP-hk-mo.hu Hiperesqueletic Leptosols (Molic, Humic), LP-hk-ca.hu Hiperesqueletic Leptosols (Calcaric, Humic), LP-rz-hu Rendzic Leptosols (Humic), GL-mo-ca.hu.ce Molic Gleysols (Calcaric, Humic, Clayic), LP-li-ca Litic Leptosols (Calcaric), PH-rz-ce Rendzic Phaeozems (Clayic), LP-rz-ce Rendzic Leptosols (Clayic). **Terrain forms**: Prbdc Well-drained and cultivated residual plain, Ccxlo Slightly undulating convex crests, Piav Wide interior plains with vegetation, Piac Wide cultivated interior plains, Ccxfo Strongly undulating convex crests, Ccxmo Moderately undulating convex tops, Piacclo Wide interior plains and slightly undulating convex crests, Ccxmlo Slightly to moderately wavy convex crests, Ccxmfo Moderately convex to strongly wavy crests, Apbdv High well-drained plains with vegetation, Alali High with slightly sloping isolated hills.

interior plains were related to the Luvisols group and they have a direct relationship to land-use: the plains with secondary vegetation (for example, the LV-ha-he.sk unit) were related to stony soils. Unlike these, the cultivated plains are not stony and are related to the LV-ni-fr.he unit. The convex LFs are related to the Leptosols group, which was previously described as shallow. Given that the slope is a determining factor in the development of the soil, the slightly-convex LFs are less stony than the LFs with a steeper slope. The units correspond to the LP-hk-rz, LP-hk.mo.hu, and LP-hk-ca.hu units.

The ADPT landscape had similar results for the convex LFs, as well as the LP-rz-hu and LP-li-ca soil units. Meanwhile, the GL-mo-ca.hu.ce soil unit is related to the Vt landform. This landform has a flat-concave, narrow, and poorly drained relief, located on the slopes of the higher elevation landscape. It acts as a drainage area that unloads in the interior plains of the EBPD landscape. Finally, the LFs of the plains and hills of the AD >200 m.a.s.l. are related to the PH-rz-hu and LP-rz-hu soil units and they are the deepest for the plain.

Legates *et al.* (2010) point out that the topological shape of the earth's surface impacts the accumulation of moisture within the soil profile. Convex LFs have low infiltration and high internal drainage, which reduces moisture within the soil profile (Sener and Oztürk, 2019; López-Castañeda *et al.*, 2017). In flat FTs, the infiltration speed and internal drainage depend on the gradient of the slope. Concave LFs have greater infiltration and lower internal drainage, which favors a longer period of humidity within the soil (Sener & Ostürk, 2019), as in the cases of marsh and fluvial-marshes landscapes.

Based on an analysis of the catenas, it can be inferred that, in sites with convex relief, the aeration within the soil profile is greater than in flat or concave LFs. In addition, the humidity conditions in the latter allow a better development of the vegetation, which influences the *in situ* development of the soil (Krasilnikov *et al*., 2011).

The quantity and variation of the geomorphological landscapes found in the toposequences of this study were greater than those registered in other states located in the same morphogenetic environment (Yucatan Peninsula). For this study, we found 23 geomorphological landscapes in Campeche, while Bautista-Zúñiga *et al*. (2015) only identified five landscapes in Yucatán and Zavala-Cruz *et al*. (2016) described 12 landscapes in Tabasco, a state adjacent to the peninsula.

CONCLUSIONS

The two catenas studied showed a strong correlation between relief and soil types, both at the geomorphological landscape level and at the landform level. The catenas provided the basis for the cartographical definition of the distribution of soils in the state of Campeche. This definition will be incorporated into a proposal for the sustainable management of soils in Mexico. By controlling the distribution of the masses and the energy of the relief zone, it was possible to distinguish each landscape's accumulation, erosion, and karstification areas. Understanding the geomorphology-soil relationships in a given region lays the foundations for the establishment of soil distribution models, which facilitates soil cartography and territorial planning.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of the harvest date, calcium and other chemicals on the quality and storability of 'Golden Smoothie' apples

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ABSTRACT

Objective: Apples cultivated in Mexico generally are smaller and softer than those produced in other geographical latitudes considered as optimal for apple production. The aim of this evaluation was determine the effect of applications with calcium, nitrogen, potassium, magnesium, sulfur and naphthaleneacetic acid (NAA), as well as the harvest date on the quality of apple fruits.

Methodology: 'Golden Smoothie' apple trees were treated foliarly with $CaCl_2$ with and without NAA, and with a mixture of N, K, Mg and S or gypsum applied to soil for two years. Apples were harvested at 141 (regular harvest date), 161 (mid-late harvest) and 171 days (late harvest) after full bloom (DAFB) and evaluated for quality at harvest time and during their storage at 0 °C for up to 179 days.

Results: Foliar applications of $CaCl_2$ significantly increased the calcium content in fruit and leaves, but fruit quality, including firmness, was not influenced. Fertilization of soil with the mixture of nutrients, including $CaSO_4$, did not influence the fruit quality. Lately harvested fruit was 14.9% heavier but 17.1% softer than fruit picked at the commercial harvest date. Delaying of fruit harvest reduced about 43 d the storability of fruit. Based in these results, the relative softness of apples grown in Mexico is not related with its calcium content, hence unlikely to be overcome with the application of this mineral.

Conclusions: Even in the control fruits, both seasons, the stored fruits do not show some physiological disorder as bitter pit.

Keywords: Fertilization, posharvest, fruit firmness, physiological disorder

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INTRODUCTION

The largest apple-producing region in Mexico is in the Chihuahua state (SIAP, 2019). This region faces some problems compromising apple quality and production. Apple (*Malus* × *domestica* Borkh.) orchards in this region are established at <30 ° north latitude, which is below of the typical latitude of the regions with the highest apple production in the world (~40 °). This causes a low cold accumulation by trees during winter, reducing fruit yield. The high fluctuation of daily temperatures in the region, frequently of 25 °C, related with the altitude of these orchards (1900 to 2100 masl), negatively affects the

Image by Couleur at Pixabay

tree dormancy and blooming, and consequently, the fruit yield and quality (Palmer *et al.*, 2003). Trees of these orchards frequently show a prolonged blooming period, which usually affect the overlap of flowering periods of pollinizers with the producer cultivars, reducing the pollinizing rate and fruit yield (Soto-Parra *et al.*, 2020). Furthermore, summer photoperiod in Chihuahua is shorter than that of regions located at latitudes ≥ 40°, limiting the vegetative and fruit growth (Lakso & Goffinet, 2013). This condition is exacerbated by the use of black nets to prevent hail damage, reducing even more the exposition of fruits and trees to sun light, reducing the photosynthetic activity and development of fruit color (Guerrero *et al.*, 2002). These adverse conditions collectively compromise all quality attributes of fruit, especially fruit firmness (Olivas, 2012). Ornelas-Paz *et al.* (2018) observed that the apples cultivated in Chihuahua were very soft, presumably as a consequence of a delayed commercial harvest date. Firmness is one the most important quality attributes of apples, determining their acceptance by consumers and storability (Hoehn *et al.*, 2003). Several cultivation practices are currently being tested by apple producers in Chihuahua to improve fruit quality, including improvements on orchard nutrition and handling of the harvest date. The nutrition of apple trees with calcium has been proposed as an alternative to increase the firmness of apples because calcium ions prevent dissociation of pectin chains and cohesion loss between cells, retarding postharvest fruit softening (Ornelas-Paz *et al.*, 2018). Calcium applications also prevent the development of physiological disorders (*e.g.* bitter pit) in apple fruit (Donahue *et al.*, 2018). Calcium reduces the respiration rate and ethylene production, delaying ripening and retarding loss of firmness (Tyagi *et al.*, 2017). However, the success of nutrition with calcium can be influenced by several factors, including the application of other chemicals and nutrients. Application of naphthaleneacetic acid (NAA), for example, can reduce vegetative growth by inhibiting photosynthesis and favors calcium accumulation in fruits (Amarante *et al.*, 2020). On the other hand, a high availability of other minerals as N, K and Mg can reduce the assimilation of calcium, and consequently promote bitter pit and other physiological disorders in apples postharvest (Jemric *et al.*, 2016), with a reduction in flesh firmness (Casero *et al.*, 2010).

Thus, the aim of the study was to determine the effect of applications with calcium, nitrogen, potassium, magnesium, sulfur and NAA, as well as the harvest date on size and firmness of 'Golden Smoothie' apples cultivated in Chihuahua, Mexico.

MATERIALS AND METHODS

Plant material. The study was conducted in 2018 and 2019, in a commercial apple orchard (cv. Golden Smoothie on MM-111 rootstock) in Cuauhtémoc, Chihuahua, Mexico (28° 34' N, 106° 54' W; 1995 masl). Trees were 35 years-old and the tree density was 775 trees·Ha^{-1}.

Treatments. Groups of five trees were selected to monitor the effect of each of the treatments showed in the table 1. Stopit™ was used as the calcium chloride source (160 g·L^{-1} Ca^{2+} w/w, Phosyn Needs plc. UK) and always was added with Solubor™ (Sodium borate 20.5% B, 20 mule team Borax™) at 1 g·L^{-1}. Fruitone N™ was used as the source of naphthaleneacetic acid (3.5% NAA, AMVAC, Mexico).

Table 1. Treatments applied on 'Golden Smoothie' apple trees. 2018 and 2019.

Treatment	Product	Dosis	Applications[y]	
			Soil	Foliar
T1	Calcium chloride (12)[z]	1.6 g $Ca^{+2}\cdot L^{-1}$		12
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	100 g/tree 100 g/tree 51 g/tree 300 g/tree	10 10 10 10	
T3	Calcium sulfate NAA	300 g/tree 0.1 $g\cdot L^{-1}$	10	5
T4	Calcium chloride (6)	5.8 g $Ca^{+2}\cdot L^{-1}$		6
T5	Calcium sulfate NAA Calcium chloride (6)	300 g/tree 0.1 $g\cdot L^{-1}$ 5.8 g $Ca^{+2}\cdot L^{-1}$	10	5 6
T6	Control	***	***	***

z.- Numbers in parentheses indicate the sprays in the year.
y.- Foliar and soil fertilizations were applied at intervals of 8 days.

Foliar and soil samplings. Due to the lack of financing in 2018, and to observe the residual effect of two years of treatments, in July 2019 soil and leaf samples were collected in triplicate for the chemical measurements. One kilogram of soil (5-20 cm depth) was taken up from both control trees and trees treated at soil (treatments 2, 3, 5 and 6), 1 and 4 treatments were not sampled since they did not receive application at soil. Per triplicate, forty leaves were taken from all experimental trees.

Harvest date. In 2018, two harvest dates were included, the commercial harvest (141 days after full bloom, DAFB) and the late harvest (171 DAFB), whereas only a mid-late date (161 DAFB) was considered in 2019. On each harvest date, 12 fruits from each experimental group (treatment) were randomly selected to be analyzed for weight, flesh firmness, total soluble solids, starch index, skin color and calcium content. From each treatment and harvest date, approximately 20 kg of fruit were stored at 0 °C. Three (2018) and two (2019) samplings of 12 fruits each were carried out during storage, which were evaluated for flesh firmness. The storage periods were 179 and 135 d in 2018 and 2019, respectively.

Physicochemical measurements. The mineral content of soil, leaves and fruits were determined in the Laboratory of the Regional Association of Fruit Growers (UNIFRUT) in Chihuahua. Nitrogen was determined by the micro-kjeldhal method (Bremner & Mulvaney, 1982). To quantify K, Ca and Mg, samples were subjected to an acid digestion (Cottenie, 1994), and their concentrations were obtained by atomic absorption spectrophotometry (Perkin Elmer, AAnalyst 100). The pH and electrical conductivity of the soil samples were measured according to the procedures described by SAMLA (2004) and Hoskins (1997), respectively. Aforementioned analyses were carried out at the end of the two evaluation years. Fruit size was determined using a vernier caliper and the weight with a digital balance (Ohaus, USA). A Universal Texture Analyzer TA-XT2i (Texture technologies Corp. USA) was used to evaluate the fruit flesh firmness, recording the maximum force to penetrate 10 mm depth with a cylinder probe (Ø=11 mm) at 10 mm·sec^{-1} in two opposite sides of each fruit. Total soluble solids content (TSS) was determined in fruit juice with a digital refractometer PAL-1 pocket (ATAGO, Japan). Skin color was evaluated on two opposed sites of each fruit with a CR-300 Chroma Meter (Minolta, Japan). Starch index was determined by immersing fruit slices in an iodine-potassium iodide solution. The incidence of physiological disorders such as bitter pit was evaluated at the same time as the rest of quality parameters, counting fruits with symptoms.

Data analysis. Each harvest date and sampling time from storage were analyzed separately, using a completely randomized design with twelve replicates (fruits) for each treatment. Data were subjected to analysis of variance (ANOVA) and means were separated by Tukey test (p=0.05) using the SAS system for Windows version 9.0 (SAS Institute Inc., Cary, NC, 2012).

RESULTS AND DISCUSSION

Harvest date. In 2018, the delayed harvest (171 DAFB) caused an increase in fruit weight of 14.9%, representing an increase in yield of ~5.8 Ton·ha^{-1} compared to the commercial harvest date (141 DAFB); however, lately harvested fruit was 12.6 N less firm (Table 2). An average fruit firmness of 60.4 N was obtained with a mid-late harvest (161 DAFB) in 2019, similar to that observed with the late harvest in 2018 (61.0 N). According to our results, Hall *et al.* (2001) found that 'Golden Delicious' apples continue growing after reaching the physiological stage required for commercial harvest (150-160 DAFB). This growing rate can be of up to 1 g per day. Our study evidenced that fruits grew at a rate of 0.74 g per day after the commercial harvest date, whereas its firmness decreased 0.42 N per day. Similar rates of fruit growing and softening were reported by Johnston *et al.* (2002). Some studies have demonstrated that delayed harvest of fruit compromise fruit quality attributes as firmness, color, starch hydrolysis, shelf life, and increase the difficulty for controlling the negative effects of ethylene biosynthesis on fruit preservation (Ornelas-Paz *et al.*, 2018).

On the other hand, the Official Mexican Standard (NMX-FF-061-SCFI-2003) establishes a minimal firmness of 44.5 N for 'Golden Delicious' apples to be marketed fresh. In this experiment, the fruits harvested on a commercial date reached this value at 103 days of storage, whereas those lately harvested did so at 60 days, which means a 58% less storability of the fruit. In terms of fruit firmness, the nutrition treatments evaluated did not influence the storage capacity of the apples (Figure 1).

Chemical treatments. $CaCl_2$ sprays effectively increased the calcium content in leaves and fruits; however, fruit quality at harvest and during storage was not influenced by these treatments (Tables 2-4, Figure 1). Moreover, 6 sprays of $CaCl_2$ at high doses (5.8 g·L^{-1}) increased the calcium content in leaves more effectively than 12 sprays at low doses (1.6 g·L^{-1}). The lowest values of calcium content in the fruit were observed for control fruit (Table 4); however, symptoms of Ca^{2+} deficiency were not observed in this fruit both at harvest and during storage. According to our results, in other assays, eight $CaCl_2$ sprays not shown beneficial effects on fruit firmness, soluble solids, ethylene production, starch index,

Table 2. Effect of the chemical treatments on the fruit quality of 'Golden Smoothie' apples at commercial and late harvests. 2018.

Treatment	Product	Weight (g)	Flesh firmness (N)	TSS (°Brix)	Starch index (1-6)	Skin color (Hue)
	Commercial harvest (141 DAFB)					
T1	Calcium chloride (12)[y]	119.6ns[z]	75.0ns	12.7 ab	4.0 a	116.5 ab
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	134.0	72.0	12.7 ab	2.9 b	116.7 a
T3	Calcium sulfate NAA	126.3	72.7	12.4 b	4.0 a	115.2 bc
T4	Calcium chloride (6)	130.3	75.5	13.2 a	4.0 a	114.6 c
T5	Calcium sulfate NAA Calcium chloride (6)	128.4	71.4	12.3 b	4.4 a	115.6 abc
T6	Control	123.7	75.0	12.7 ab	3.9 a	115.5 abc
	Late harvest (171 DAFB)					
T1	Calcium chloride (12)	145.1 ab	57.4ns	14.3 ab	6.0ns	108.1ns
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	167.1 a	59.7	13.7 abc	6.0	109.5
T3	Calcium sulfate NAA	136.6 b	64.7	14.6 a	5.9	111.8
T4	Calcium chloride (6)	147.0 ab	59.8	13.2 abc	5.9	109.6
T5	Calcium sulfate NAA Calcium chloride (6)	144.4 ab	63.4	12.6 c	6.0	111.6
T6	Control	156.0 ab	60.8	13.0 bc	5.9	108.7

z.- Mean separation by Tukey's test ($p \leq 0.05$). Means followed by different letters within the same column and time of harvest are significantly different. DAFB: days after full bloom, ns: not significant.
y.- Numbers in parentheses indicate the sprays in the year.

and peel color, however the incidence of bitter pit in postharvest was reduced (Hoying & Cheng, 2013). Ornelas-Paz *et al.* (2018) demonstrated that the changes of Ca^{2+} content in 'Golden Delicious' apples did not correlate with fruit firmness. However, other studies have shown variable effects, year to year, of the calcium treatments on the fruit quality (Casero *et al.*, 2010); even some researchers suggest that soil applied calcium can replace the foliar application traditionally used in apple orchards (Danner *et al.*, 2015). Sometimes, calcium sprays do not increase the fruit firmness at harvest, however, the softening in storage is delayed, probably due to a reduction in the respiration rate and senescence of fruit (Xu *et al.*, 2022). The efficacy of the calcium sprays can be related with the weather conditions, since in years with low precipitation and high air temperature the calcium effect could be useless or adverse (Moor *et al.*, 2006). Moreover, Danner *et al.* (2015) found ineffective the sprays with calcium in an apple orchard located at 26° 34' S, similar latitude to those of our experimental condition (28° 29' N).

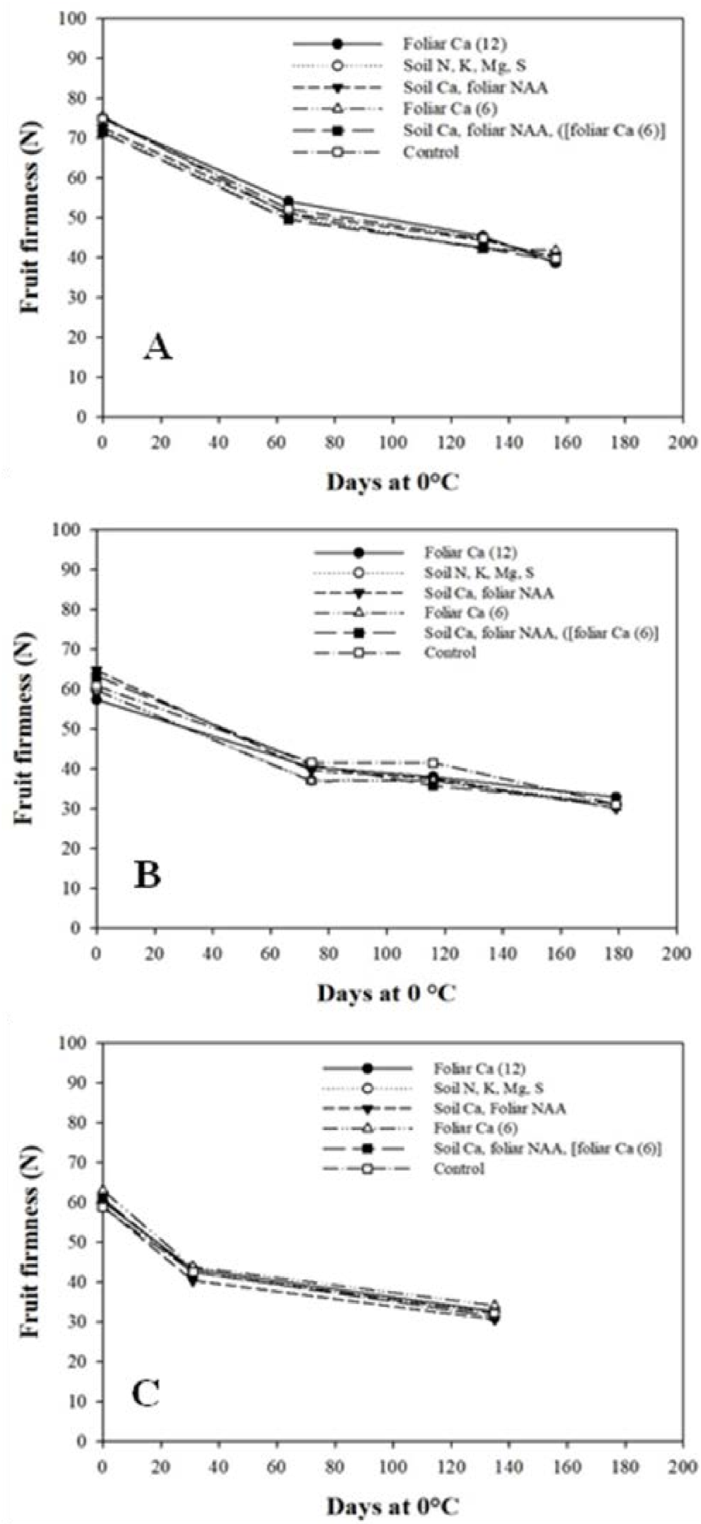

Figure 1. Flesh firmness of fruits 'Golden Smoothie' during the storage at 0° harvested at: A) 141 DAFB (commercial date), B) 171 DAFB (late date) in 2018; and C) 161 DAFB (mid-late) in 2019.

Table 3. Effect of calcium application on the fruit quality of 'Golden Smoothie' apples at mid-late harvest. 2019.

Treatment	Product	Weight (g)	Flesh firmness (N)	TSS (°Brix)	Starch index (1-6)	Skin color (Hue)
	Mid-late harvest (161 DAFB)					
T1	Calcium chloride (12)[y]	122.7ns[z]	60.2ns	12.4 ab	5.7ns	112.6ns
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	125.7	60.5	12.2 ab	5.9	113.3
T3	Calcium sulfate NAA	127.3	59.1	13.1 a	5.7	113.4
T4	Calcium chloride (6)	116.5	62.9	12.0 b	5.9	113.2
T5	Calcium sulfate NAA Calcium chloride (6)	125.8	60.8	12.3 ab	5.8	111.6
T6	Control	121.5	58.9	12.1 b	5.8	114.3

z.- Mean separation by Tukey's test ($p \leq 0.05$). Means followed by different letters within the same column and time of harvest are significantly different. DAFB: days after full bloom, ns: not significant.
y.- Numbers in parentheses indicate the sprays in the year.

Table 4. Effect of the treatments on the calcium content in 'Golden Smoothie' apple trees. 2019.

Treatment	Product	Ca (%)	
		Foliar	Fruit
T1	Calcium chloride (12)[y]	2.1 abc[z]	0.92 a[z]
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	1.8 bc	0.77 a
T3	Calcium sulfate NAA	1.9 bc	0.88 a
T4	Calcium chloride (6)	2.3 ab	0.96 a
T5	Calcium sulfate NAA Calcium chloride (6)	2.6 a	0.98 a
T6	Control	1.5 c	0.49 b

z.- Mean separation by Tukey's test ($p \leq 0.05$). Means followed by different letters within the same column are significantly different.
y.- Numbers in parentheses indicate the sprays in the year.

In this experiment, the inefficacy of calcium sprays to improve the fruit quality, especially on firmness, could be attributed to the calcium supplied via xylem previous seasons and stored in wood and bark, preventing the visualization of the effects of the treatments. Besides that, control fruit, without calcium applied, even so did not show symptoms of disorders as bitter pit. On the other hand, Hocking *et al.* (2016) found that the calcium absorbed by the roots moves very slowly through the tree to the fruit, with this process taking up to years.

In our study, NAA did not influence the fruit quality and calcium content, being that it has demonstrated that NAA decreases photosynthesis, promotes dark respiration, inhibits the transport of photosynthates to fruits, and consequently vegetative growth is decreased, promoting the calcium availability for fruits (Amarante *et al.*, 2020).

The treatment with a mixture of nutrients at soil decreased it's pH from 7.6 to 6.4, effect largely caused by the sulfur added. However, an effect on the Ca^{2+} content in fruits, leaves or bitter pit development, were not observed, as has been suggested by Cheng (2015). Moreover, this mixture increased the electrical conductivity of soil to 4.5 $ds \cdot m^{-1}$ as well as its content of NO_3^-, P, K^+ and Mg^{2+} (Table 5). Although studies have demonstrated that an overfertilization on apple trees with N, K^+ or Mg^{2+} can induce a calcium deficiency in fruits (Jemric *et al.*, 2016), the fertilization in our experiment with N, K^+, Mg^{2+}, S and Ca^{2+} did not influence the calcium content in leaves or fruits as well as the fruit quality.

CONCLUSIONS

Foliar and soil applications with calcium in the apple orchard increased the content of this mineral in leaves and fruit but did not influence fruit quality in both years evaluated, including firmness. Delayed harvest of fruit favored fruit size but compromised fruit firmness. The storability of the fruit lately harvested was 43 d less than that of fruit harvested at the commercial date. Even in the control fruits, both seasons, the stored fruits do not show some physiological disorder as bitter pit.

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Table 5. Effect of the treatments applied to soil over the mineral content and chemical properties of the soil. 2019.

Treatment	Ground treatment	Nitrates ($Kg \cdot Ha^{-1}$)	K (ppm)	Ca (ppm)	Mg (ppm)	pH	EC (ds/m)
T2	Urea Potassium sulfate Magnesium sulfate Wettable sulfur	371.8 a[z]	1,683.0 a	5,178[ns]	448 a	6.4 c	4.5 a
T3	Calcium sulfate	57.6 b	1,024.2 b	5,266	396 b	7.4 b	2.0 b
T6	Control	47.8 b	1,189.3 b	4,942	434 a	7.6 a	1.4 b

z.- Means followed by different letters within the same column are significantly different according to Tukey's test ($p \leq 0.05$). ns, not significant.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Feasibility of the adoption of soil erosion mitigation technology on farmland and pastures in northern Mexico

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ABSTRACT
Objective: To identify the socioeconomic factors which determine the adoption of soil erosion mitigation technologies in the Nazas-Aguanaval watershed region in the state of Durango, Mexico.
Design/methodology/approach: During 2018, 61 semi-structured surveys were applied to farmers in the region. The variables associated with the willingness to adopt or not were analyzed with a maximum likelihood binomial Logit regression model.
Results: Perception of the soil erosion problem, location of the watershed or agricultural unit, and economic activity were the most influential variables in the model. The main variable that conditions the willingness to adopt technologies to improve the soil is the perception of soil erosion in production areas, with a marginal effect of 45.03%.
Limitations on study/implications: The results of this survey may only be applicable to the study area.
Findings/conclusions: Training is necessary to promote and increase the perception, understanding and acceptance of soil erosion mitigation technologies.

Keywords: Natural resource economics, environmental impact, logit regression, environmental sociology.

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INTRODUCTION

Validation, transfer and adoption of technologies for natural resource management and productivity are complex processes that require interdisciplinary participation in order to have a greater impact on regional development (Borja-Bravo *et al.*, 2020). The accelerating degradation process of natural resources due to anthropogenic practices and the resulting environmental impact pose new challenges beyond the creation of technology; they also require its application by users who intervene in the production chain (Cottler *et al.*, 2007).

Soil erosion is one of the most frequent and important degradation processes of natural resources and implies a complex process of soil particle detachment, movement and deposition, mainly by rainwater and wind (Bolaños *et al.*, 2016). In the past few decades, different soil management practices have been evaluated with the goal of mitigating soil erosion, not only because of the repercussions of a decrease in profitability, but also in order to improve ecosystem services. Management practices such as: rainwater catchment and soil moisture retention systems (Cruz-Martínez *et al.*, 2016); use of water retaining agents and pasture restoration (Yáñez-Chávez *et al.*, 2018); use of cover crops (Huerta-Olague *et al.*, 2018); and tillage systems (Velásquez-Valle *et al.*, 2015).

Also, there is a wide variety of technologies that can be considered conservation practices, which can maintain or improve soil fertility and reduce erosion (Soule *et al.*, 2000). Technical innovation includes presenting new ways of using an established technology, which can be subject to social processes that determine its adoption or adaptation to it or, ultimately, its rejection (Ruíz *et al.*, 2006). This study's objective was to identify the socioeconomic factors that determine the adoption of soil erosion mitigation technologies for better decision-making towards the improvement of soil management in agricultural lands.

MATERIALS AND METHODS

Geographic location of the study area. The study was conducted in the municipalities of Mapimí, San Pedro del Gallo and San Luis del Cordero, which are part of the middle and lower Nazas-Aguanaval watershed in the state of Durango, Mexico (23° and 27° N and 106° and 102° W) at an altitude of 1,176 m, with average rainfall of 304 mm, and a maximum recorded temperature of 44 °C and a minimum of 10.2 °C (Medina, 2005).

Sample size. To collect the necessary information, a survey was designed with a total of 28 questions grouped into four sections: tenure and land use; soil management carried out by producers and perception of the erosion problem; willingness to adopt soil erosion mitigation technologies; and, socioeconomic conditions of the subject population (WOCAT, 2016).

The target population was predominantly livestock farmers registered with each municipality's Center for Rural Development Support (Centros de Apoyo al Desarrollo Rural, CADER). The size of the sample was calculated with the formula for finite population, when the total number of observation units that form it is known (Aguilar-Barojas, 2005):

$$n=\frac{N+Z_x^2+p+q}{d^2\left(N-1\right)+Z_x^2+p+q}$$

Where, n=sample size; N=total population (580); Z=confidence level (1.645); p=probability of success or expected proportion (0.5); q=probability of failure ($1-p$); and d=accuracy, referring to the maximum admissible error in terms of proportion (0.1).

The sample sizes calculated were 19.26 and 18 producers in the Mapimí, San Pedro del Gallo and San Luis del Cordero municipalities, respectively.

Econometric model. In regards to the willingness of the producer to invest or not in soil improvement practices, the answer is dichotomous: 0 in case of a negative response and 1 in case of a positive response. Based on this, a regression model with a cumulative logistic probability function is assumed (Haab & McConnell, 2002):

$$Pr(si) = \frac{1}{1+e^{-x}}$$

Where, *Pr*=Probability of adoption of the soil erosion mitigation technology, which was estimated using the maximum likelihood method.

Variables measured. The variables measured were: willingness to adopt the technology, with a value of 0 in the case of a negative response and a 1 in the case of a positive response (dichotomous); main economic activity of the producer, where: 1=agriculture, 2=livestock production and 3=mixed; type of land tenure, where: 1=owned, 2=rented, 3=mixed; perception of erosion: 1 (yes), 2 (no); management, where 1=implements some type of practice, 2=no practice is implemented, and 3=is unaware of any type of practice.

Data analysis. To estimate the regression parameters, the Statistical Analysis System (SAS) software was used; to calculate the margin of error, NLOGIT 4.0 was utilized. A binomial Logit regression model was used since it is the most appropriate method for this type of study (Tadesse & Belay, 2004; Calatrava *et al.*, 2007; Mekuriaw *et al.*, 2018).

RESULTS AND DISCUSSION

Land tenure and use. For the majority of producers (89.7%), the land tenure is private property and only 3.3% rent the land, while 6.8% of the land tenure is mixed: owned and rented. In the cases where the surveyed parties were owners, 13.5% declared the land as smallholding, 76.9% as ejido land, while 3.8% as communal land and the remaining 5.8% as a combination of *ejido* and communal land. Of the farmers, 77.6% carry out both agricultureal and livestock production activities, 10.3% carry out agricultural activities exclusively, and 12.1% exclusively livestock production (Figure 1).

The type of land tenure system is revealed as the determining variable in the relationships that impact soil erosion mitigation practices. Udayakumara *et al.* (2010) indicate that security in land tenure has a positive effect upon the decision of farmers to invest in soil conservation methods. Soule *et al.* (2000), report that the impact of land tenure can depend on the time and magnitude of costs and yields generated by the practice of soil conservation. For agricultural activity, 72.5% of the people surveyed carry out their activities on less than 15 ha, 21.7% on 19 to 40 ha, while 5.8% carry out their activities on more than 41 ha. The main crops are corn, oats and different species of grasses, for which 87.9% are rainfed, 5.2% are irrigated with well water, and 6.9% use a combination of irrigation and rain water. In regards to livestock production, 50% of those surveyed carry out their activities on less than 18 ha, 29.2% on 19 to 66 ha, 4.2% on 67 to 150 ha, 8.4% on 151 to 200 ha, 6.1% on 201 to

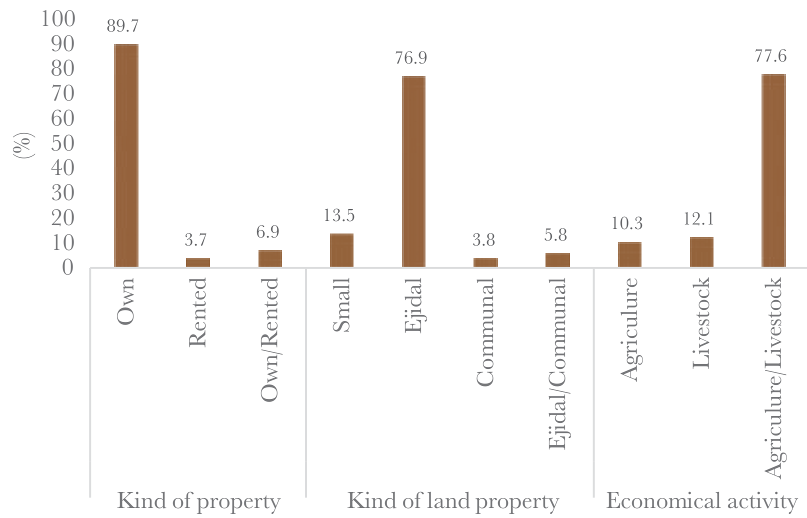

Figure 1. Land tenure type and system and economic activity of the surveyed farmers in the municipalities of Mapimí, San Pedro del Gallo and San Luis del Cordero in the state of Durango.

500 ha, and only 2.1% on more than 500 ha (Figure 2). The vast majority of producers raise cattle (96%) and only 4% raise ovine and caprine livestock, where 30% are self-sufficient in regards to fodder; 17.3% use pastureland and 17.3% produce and purchase fodder. The rest of the livestock producers use a combination of pasturing, production, and purchase of fodder.

The size of the agricultural production units demonstrates a decisive relationship to soil conservation. The possible explanation is that the larger units can be associated with greater wealth and availability of capital, which increases the probability of investment in soil conservation practices (Mekuriaw *et al.*, 2018). However, Soule *et al.* (2000) clarify that the size of the plot of land can have different magnitudes of correlation when each type of tenure is analyzed separately.

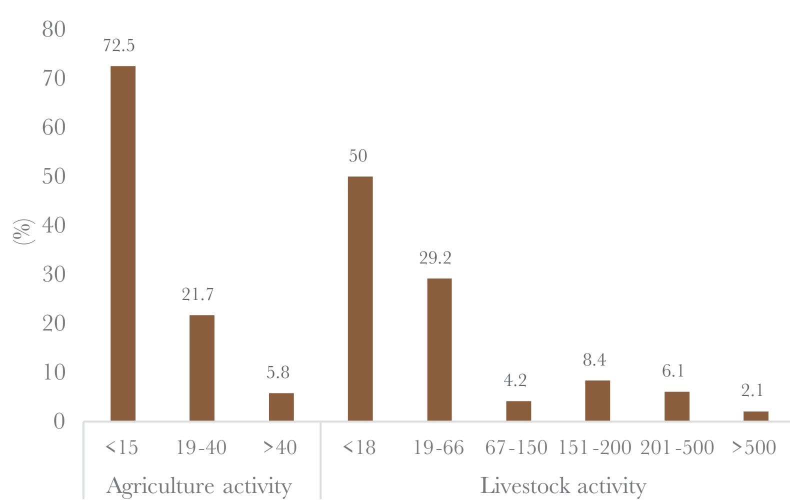

Figure 2. Land extension in hectares by type of economic activity carried out by farmers of the Mapimí, San Pedro del Gallo and San Luis del Cordero municipalities in the state of Durango.

Perception of the soil erosion problem and management practices. Of the respondents, 71.5% do recognize that the soil is eroded by sediment dragging due to torrential rains which result in the increase of exposed ground and lack of topsoil, turning it into unproductive land; however, 26.6% are unaware of this problem. Within this framework, 71% affirmed implementing some type of soil management practice to mitigate the problem, while 27.4% denied implementing any type of management and the remaining 1.5% declared being unaware of any kind of practice of this nature. The building of mounds or levees is the most generalized soil management practice (83.3%), while only 2.4% practice reforestation and 14.3% mention carrying out other practices. Of the surveyed population, 66.7% associate the soil erosion problem to rainwater runoff; 9.5% to the effects of the wind; 16.7% to the combined effects of rainfall and wind; and only 2.4% to animal overload.

By analyzing the perception of the soil erosion problem, regardless of its cause, the study identified that a vast majority of the producers perceive that this process affects productivity negatively. According to Hammad & Tumeizi (2012), the perception of soil erosion is seen as a consequence of natural factors, besides anthropogenic practices, and in any case as having negative economic impacts on production. If soil erosion reduces agricultural benefits, it is more likely for conservation practices to be adopted (Calatrava *et al.*, 2007).

The location of the watershed, the willingness to accept technology and the perception of erosion were the only variables significantly different from zero ($P \leq 0.05$) (Table 1). As such, we can say that the variables that influence the adoption of technologies for environmental damage mitigation are given by the geographic location (watershed), the economic activity and whether or not there is the perception that the plot of land is affected by environmental factors. Tadesse and Belay (2004) agree that the awareness of the farmers regarding the soil erosion problem and the location of the agricultural land within the watershed influence positively the probability of adoption of soil conservation technologies.

The results of the econometric model regarding the willingness of producers to learn techniques that mitigate erosion are justified by the fact that the cost of these conservation practices surpasses the short term benefits; the benefits of soil conservation practices are gained over a longer term, while the costs of conservation practices occur in the short term (Calatrava *et al.*, 2007).

Regarding the willingness to invest a minimum in the development and adoption of technologies that mitigate the negative effect of soil erosion affecting productivity, a positive response by 74.13% of those surveyed was found, while 25.86% responded as unwilling.

Table 1. Estimated parameters for different variables related to the willingness to adopt soil erosion mitigation technologies.

Parameter	FG	Estimator	Standard error	Chi-squared of Wald	Pr>ChiSq
Intercept	1	−8.7013	3.0226	8.2870	0.0040
Location of watershed	1	2.9734	0.9650	9.4932	0.0021
Availability of acceptance	1	1.2724	0.6132	4.3061	0.0380

Marginal effects. The main variable that affected the decision to pay for technology adoption was the producer's awareness of the existence of soil dragging due to wind or rain. The probability of paying to implement soil improvement techniques increased by 45.03% when farmers are aware of the problem on their lands, while the decision to adopt increases by 41.57% when the production unit is located in the middle watershed (Table 2). This indicates that soil erosion is perceived more in the middle watershed and less in the lower watershed, given that the second is where soil deposition takes place.

The willingness to pay that was observed in this study is consistent with evidence reported by other authors (Tadesse and Belay 2004, Mekuriaw *et al*., 2018), who affirm that the awareness of the farmers about the soil erosion problem, its costs and benefits, is key to determine the adoption of soil conservation practices.

Finally, the economic activity or, ultimately, the profitability of the production unit also influenced the model positively. The producers that combine activities (agriculture and livestock production) have a 17.78% greater probability of implementing erosion mitigation technologies compared to those who only carry out one activity in their plots. If we assume that the farmer's profits increase when their costs decrease by producing their own forage for the livestock, then the additional income can be invested in soil improvement.

Amarasekara *et al*. (2009) point out that producers tend to invest more in soil conservation measures when there is an increase in farming income; however, farming income is determined by many other technical and socioeconomic factors such as type of crop, education level, size of the unit, among others. Soule *et al*. (2000) mention that farmers consider economic factors in order to decide whether or not to adopt specific conservation practices. These factors include short term profitability, as well as the long term value of the assets.

CONCLUSIONS

Understanding and accepting technology is important even when the financial yields are reflected in the mid-term, since eventually the investments will generate a sustained profit, thus compensating for the short-term increase in production costs. Any program used to mitigate the impact of soil erosion must take into account the socioeconomic characteristics, the location of the plots of land, the perception of the problem, and above all, the technology must be suitable to the context of the producers. Training in the methods of soil degradation management is necessary for a successful intervention.

Table 2. Estimated marginal effects according to the variable analyzed.

Variable	Marginal effect
Location of watershed	0.41571
Technology acceptance	0.17789
Erosion perception	0.45032

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AP AGRO PRODUCTIVIDAD

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Agricultural rotation crops: adaptive strategies of two farming communities in Champotón, Campeche

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ABSTRACT

Objective: To identify the agricultural rotation crops grown throughout a year by the producers of two farming communities in Champotón, Campeche, Mexico.

Design/Methodology/Approach: This exploratory-descriptive research was conducted in 2019, in Santo Domingo Kesté and Sihochac, Champotón, Campeche. Documentary research was conducted and a survey with a total of 200 questionnaires was applied, following the snowball technique. The resulting data were analyzed with descriptive statistics using Excel's statistical package.

Results: Chihua pumpkin (*Cucurbita argyrosperma* Huber) and sugarcane (*Sacharumm officinarum*) are the most economically important crops. The former is specific to Santo Domingo Kesté and the latter, to Sihochac. Maize is grown in May in Sihochac, and in different months in Kesté. Chihua pumpkin, peanut (*Arachis hypogaea*), bean (*Phaseolus vulgaris*), sesame seed (*Sesamum indicum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), and hibiscus (*Hibiscus sabdariffa*) are grown only in Kesté, in different times of the year.

Study limitations/Implications: Since this research is of a local nature, its results cannot be generalized, although they may be similar to other regions of the country.

Findings/Conclusions: Some agricultural relay crops are grown in Kesté and not in the Sihochac community. The way in which each community organizes its crops depends on socio-cultural factors and available resources.

Key words: Rotation crops; Agriculture; Survey; Santo Domingo Kesté; Sihochac.

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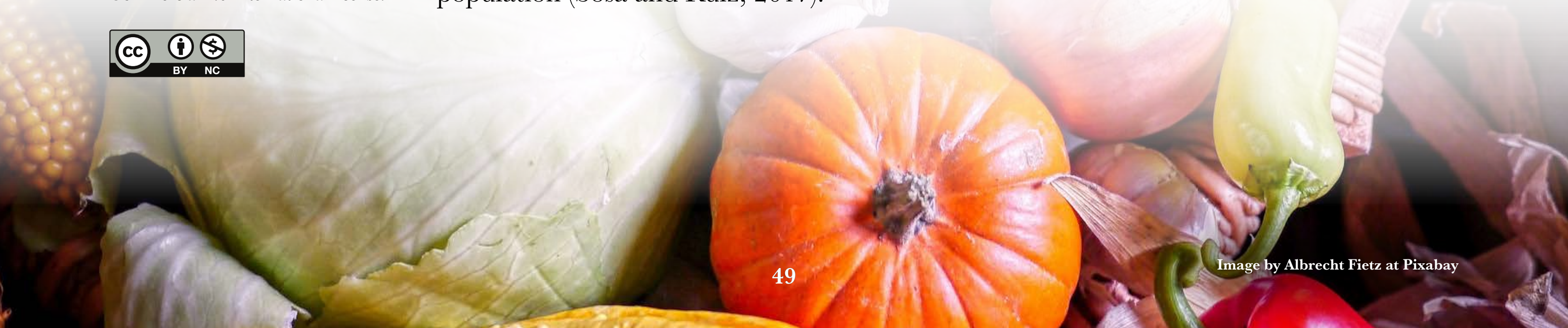
INTRODUCTION

Over the last ten years, the Mexican population grew by 13.7 million inhabitants; currently, Mexico has a population of 126 million (INEGI, 2020). Meanwhile, the agricultural surface grew by 22%: from 17.99 million ha in 1980 to 22.2 million ha in 2015. However, the available agricultural land per capita decreased by 31%. The scenario for 2050 is even more alarming. Considering a population of 140 million for the said year, the available agricultural land could diminish to 0.16 ha per inhabitant per year. Agriculture will face its greatest challenge: meeting the food supply needs of an ever-increasing population (Sosa and Ruiz, 2017).



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Food security is important in this scenario, but it is even more relevant in these times of pandemics and the COVID-19 health crisis. Consequently, it is necessary to ensure that agriculture will continue on all levels, fulfilling its role as the main source of food, as a source of energy, and as a factor in the adequate development of humankind (Luque-Zúñiga *et al*., 2021).

Therefore, as posited by Rojas *et al*. (2014), the producers' strategies are part of a greater reproduction system that has a social, economic, cultural, and environmental nature. Previous research has shown that, by way of crop diversification, producers use their abilities and potentialities as a survival strategy; this mainly includes the growth of maize, bean, wheat, alfalfa, tomato, barley, sorghum, oat, jalapeño, and onion (Sosa and Ruiz, 2017), in order to obtain more profitable and sustainable agricultural produce (Caicedo *et al*., 2020).

Moreover, multiple advantages can be gained from crop rotation —different crops succeed each other in the same soil throughout time (Silva *et al*., 2015)—, including: production sustainability; a better use of the land; positive effects on organic matter, soil structure, and erosion; fight against disease and pests; and nutrient availability, among other things (Silva *et al*., 2015).

Particularly, the State of Campeche, Mexico, is characterized by the production of different crops. Maize is the most important crop (65.9%), followed by Chihua pumpkin (14.4%) and cowpea (7.1%) (Morán *et al*., 2020). Most farmers practice family farming which, at the same time, are production and social reproduction units (Jiménez-Barbosa *et al*., 2019).

Therefore, the objective of this research was to identify the agricultural rotation crops grown in one year by producers in two rural communities of Champotón, Campeche, as a survival strategy within a context of economic-productive fluctuations, resulting from their own needs, as well as the demands of the local and regional markets.

MATERIALS AND METHODS

Location of the study area

The research was conducted in 2019, in Santo Domingo Kesté and Sihochac, two communities in the municipality of Champotón, located in the center of the State of Campeche (17° 49' and 19° 41' N; 89° 32' and 91° 08' W). Champotón borders with the municipalities of Campeche and Hopelchen to the north; the municipality of Escárcega to the south; the municipality of Calakmul to the east; and the municipality of Carmen and the Gulf of Mexico to the west. It has a territorial extension of 6,856.04 km^2 and an altitude of 27 m.a.s.l. The climate is warm sub-humid, with an annual average temperature of 26 °C (INEGI, 2020).

This research was exploratory and descriptive. Documentary research was conducted in order to get acquainted with part of the history of the studied communities. Most of the results were obtained through a survey consisting of 100 questionnaires for each of the communities, with a total of 200 questionnaires. The participants in the in-depth interviews were chosen based on a random sample using the "snowball" technique (Taylor and Bogdan, 1987), and took part freely in the study. The sample size was heuristically established at the moment when the information became redundant (Baker and Edwards, 2013). The survey results were analyzed by means of descriptive statistics using Excel's statistical package.

RESULTS AND DISCUSSION

Santo Domingo Kesté: a territory with history

To study the agricultural transition of Santo Domingo Kesté, we had to consider the age of its territory and its main historical processes. This town was founded by Guatemalan refugees who fled from the war in their country during the 1980s. They traversed the Guatemalan rainforest and crossed the Mexican border until they reached Chiapas. Subsequently, the Mexican government relocated them to different points of Campeche. A group from Huehuetenango and Quiché, in Guatemala, settled in a place they named Santo Domingo Kesté. In 1994, the UNHCR (United Nations High Commissioner for Refugees) registered 11,010 Guatemalans in Campeche who received support from the international community, several non-governmental organizations (NGOs), and ecclesiastical entities. The aid was allocated to health, education, and sustainable development projects that aimed to achieve self-sufficiency among refugees and their integration to the local society (CCBNB, BSA) (Brito, 2013).

By 2006, the state government counted a total of 3,760 inhabitants in this place; 1,360 of them had become Mexican citizens, 140 kept their Guatemalan citizenship, and 2,260 had already been born in Mexican territory (Brito, 2013). By 2013, Santo Domingo Kesté had a total of 3,763 inhabitants; 1,862 were women and 1,901 were men, which indicates that the total population remained stable during the past seven years (Brito, 2013). Currently, there are 4,461 inhabitants: 2,219 women and 2,242 men (INEGI, 2020).

The average age of the interviewees in Santo Domingo Kesté was 48.77 years; 42% had barely completed primary school. Santo Domingo Kesté had a larger percentage of people with no schooling than the Sihochac community. In this regard, Rosales *et al.* (2019) also reported differences in schooling among the populations of both communities. The interviewees in Santo Domingo Kesté have more family members (5.55) and less land to work (5.43 ha). It is necessary to mention that, when the government granted political asylum to these refugees, it only allocated each family 3.5 ha to work (Rosales-Martínez *et al.*, 2019). However, as the years have gone by, the inhabitants of this community have sold and bought land among them. The community of Santo Domingo Kesté works more daily hours than the community of Sihochac (Table 1).

Table 1. Socioeconomic variables of the interviewed producers.

Community	E	ES	IF	ST (ha)	DT	HT	TC
Sihochac	53.33	Elementary education: 58; Secondary education: 22; High school: 18; bachelor's degree: 5 and without studies: 3	3.77	6.75 ha	6.19	6.16	1.16
Santo Domingo Kesté	48.77	Elementary education: 42; Secondary level: 19; High school: 6; Bachelor's degree: 3 and without studies: 32	5.55	5.43 ha	5.67	6.95	2.31

A (E)=Age; S (ES)=Schooling; FM (IF)=Family members; TS (ST)=Total surface; WD (DT)=Worked days; WH (HT)=Worked hours; TC=Total of crops.

Sihochac

Currently, Sihochac has 2,756 inhabitants: 1,353 men and 1,403 women (INEGI, 2020). Although Sihochac is a neighboring community of Santo Domingo Kesté, its population's culture is different. This affects both their lifestyles and the way they manage their agroecosystems. Most of the population is native to Campeche. Their main agricultural crop is sugarcane, with at least 1,200 producers belonging to the Unión Cañera de Sihochac (Sihochac Sugarcane Union), which is divided into five groups: Cañeros de Sihochac, Roque Espinoza, Benito Juárez, Nueva Manera, and Unión y Libertad (Head of the CNC; personal communication). These groups deliver their production to the La Joya Sugarcane Mill, which is located in the La Joya community. The production of sugarcane is the most important agricultural activity in this community.

In the sugarcane industry, a contract is established between the producer and the mill. The production of sugarcane thereby becomes an alternative for the producers to achieve their collective goals, the most immediate of which would be developing their activity and commercializing their crop at a determined price —this differs from "unprotected" vegetable crops. However, these producers must adapt to the quality needs and controls, as well as to the planting and harvesting times established by the sugarcane mill (Parral, 2015). The average age of the interviewees in Sihochac was 53 years and they had barely completed primary school. The average number of family members is 3.77 and families have more land to work (6.75 ha) than Santo Domingo Kesté. The interviewees reported working 6.19 days per week and 6.16 hours per day (Table 1).

Agricultural activities carried out throughout a year in Sihochac and Santo Domingo Kesté

Table 2 shows that both communities grow various crops throughout the year; some of them are even grown during the same period. Defumier (1990) explains that this happens when market conditions are favorable for product commercialization. Farmers might be interested in specializing their agricultural system according to each region's comparative advantages. Nevertheless, this cannot be applied to every region. The crops in Sihochac and Santo Domingo Kesté, for instance, are grown both for self-consumption (43%) and for sale (57%), and the percentage varies according to the specific crop. For example, almost 100% of Chihua pumpkin and sugarcane is commercialized.

Meanwhile, although maize (*Zea mays*) is grown in both communities, Sihochac only grew it in May, while Santo Domingo Kesté grew it in different times of the year: on January, February, August, and September (*i.e.*, the rainy season), since this community practices rainfed agriculture (Uzcanga *et al.*, 2015). However, on more drastic occasions, producers change the land use, based on factors such as low prices of produce (at that moment the price of maize remained 5 pesos per kg), increased prices of agrochemical products, and the presence of pests and disease (Casanova-Pérez *et al.*, 2019).

Another economically important crop that stands out in Santo Domingo Kesté is the Chihua pumpkin (*Cucurbita argyrosperma* Huber), with a yield of 500 kg/ha. Chihua seeds are washed and home-dried to be sold mainly by retailers and/or local traders, with no commercialization mechanisms in the regional and national markets (Ireta-Paredes *et al.*,

Table 2. Crop sowing calendar for Santo Domingo Kesté and Sihochac.

Crop	Kesté	Sihochac	Es* kesté	Es* sihochac
Corn (*Zea mays*)	X	X	Late January, early February, August-September	May
Chihua Pumpkin (*Cucurbita argyrosperma* Huber)	X		May-June	NC
Sugarcane (*Sacharum officinarum*)		X	NC	June-July, August, September-October
Peanut (*Arachis hipogaea*)	X		May, June, August, after the corn	NC
Hibiscus (*Hibiscus sabdariffa*)	X	X	May, July, August, September, November	Not specific
Beans (*Phaseolus vulgaris*)	X		August, September	NC
Sesame seed (*Sesamum indicum*)	X		July	NC
Sweet potato (*Ipomoea batatas*)	X		May	NC
Jicama (*Pachyrhizus erosus*)	X		May	NC
Cedar (*Cedrela odorata*)		X	NC	June-July
Fruit trees	X	X	All year	All year
Cassava (*Manihot esculenta*)	X		May	NC

X=Grown in the community; SS* (ES*)=Sowing season; NG (NC)=Is not grown; Fruit trees (Frutales)=includes mango, mammee, soursop, and bananas.

2018). The price of this seed fluctuates between 25 and 30 pesos per kg. This community grows other types of crops in smaller areas and in other times of the year. Examples of this are peanuts (*Arachis hypogaea*), beans (*Phaseolus vulgaris*), sesame seeds (*Sesamum indicum*), sweet potatoes (*Ipomoea batatas*), cassava (*Manihot esculenta*), and hibiscus flowers (*Hibiscus sabdariffa*). Sugarcane (*Sacharumm officinarum*) is only grown in Sihochac; it is the main economic crop for families in this community. It has an average yield of 60 t/ha and a price per ton that fluctuates between 700,000 and 800,000 pesos. This product is delivered to the La Joya sugarcane mill, located in the La Joya community.

The sowing date is one of the most important decisions that producers must make at the beginning of each agricultural cycle. This is particularly important for seed producers, since several factors directly and indirectly affect the yield of the different varieties (Noriega-Carmona *et al.*, 2019). In these dynamics, we recorded an average of 2.31 and 1.16 crops for Santo Domingo Kesté and Sihochac, respectively. In both Sihochac and Santo Domingo Kesté, the percentage of fruit trees is lower and most of the time there are only two or three individuals in the backyard gardens. However, these trees are important for the diet of rural families, since they provide affordable fruits and spare the families from having to travel in order to buy these products in the market.

Income per agricultural crop

The crops that generated the highest income were sugarcane (41%) and Chihua pumpkin (36%). The former is only grown in Sihochac, while the latter is exclusive of Santo Domingo Kesté (Figure 1).

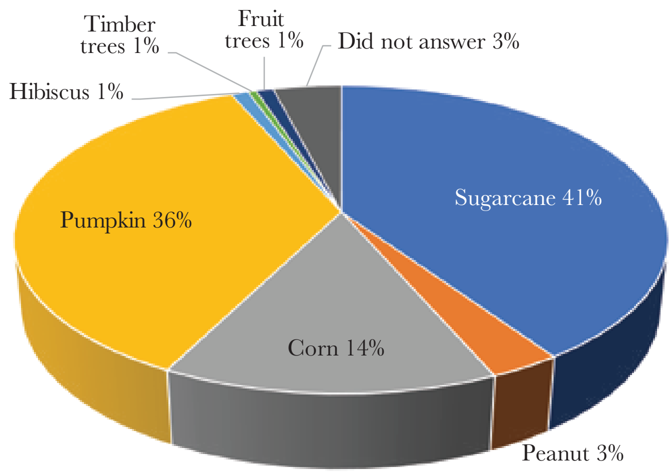

Figure 1. Economic importance per crop in the communities of Santo Domingo Kesté and Sihochac, Champotón, Campeche, Mexico.

When asked why they grow more than one crop a year, Santo Domingo Kesté's producers explained that, in first place, they do it to support their families (39%), both by selling products (economic reasons) and providing food for self-consumption throughout the year; second, because crops produce income (21%); third, to make good use of their land (8%), for food and consumption (7% each), to rent the land (6%), did not answer (5%), to make the most of the rainy season (4%), because they like it (2%), and finally because it is a habit or family tradition (1%).

CONCLUSIONS

Chihua pumpkin (*Cucurbita argerysperma* Huber) and sugarcane (*Sacharum officinarum*) are the two economically relevant crops of these communities. The former is exclusive of Santo Domingo Kesté and the latter of Sihochac. The way in which each community organizes how it grows its crops and commercializes its produce depends on the socio-cultural factors of their hometowns and the available resources. Therefore, it is necessary to draw crop rotation and implementation strategies that befit the geographic, cultural, social, and economic characteristics of rural communities such as Sihochac and Santo Domingo Kesté.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Water quality in an aquaponics system interconnected with a biofilter

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ABSTRACT

Objective: To determine the water quality of an aquaponics system interconnected by a biofilter, using sponge gourd (*Luffa cylindrica*) as an inert support.

Design/Methodology/Approach: The organisms used in the aquaponics system were juvenile tilapia (*Oreochromis niloticus*) and wormseed (*Chenopodium ambrosioides*). The following physicochemical parameters of the water were analyzed: temperature, pH, dissolved oxygen, electrical conductivity, NH_4^+, NO_2^-, and NO_3^-. Data generated in this work were subjected to an analysis of variance (ANOVA) and to the comparison of means (Tukey's test, $p<0.05$).

Results: Recirculating tank water through the biofilter and plants reduced NH_4^+ and NO_3^- by 31.6% and 18.5%, respectively. The total ammonia nitrogen in the tank did not exceed 0.022 mg L^{-1}. The fish survival rate was 100% and 725.8 g of wormseed were harvested. The wormseed did not show symptoms of mineral deficiency.

Study Limitations/Implications: Aquaponics production is still limited to small surfaces, as a consequence of the costs involved in its handling.

Findings/Conclusions: Water quality parameters of the tilapia (*Oreochromis niloticus*)-wormseed (*Chenopodium ambrosioides*) aquaponics system —interconnected through a biofilter with *Luffa cylindrica*— fulfilled the recommendations for such system.

Keywords: biofilter, aquaculture, mineralization, ammonia.

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INTRODUCTION

Aquaculture is an activity that depends on water availability. However, as a result of such activity, water becomes saturated with fish excreta and food waste (Martins *et al.*, 2009). Therefore, the exchange of water is essential; otherwise, fish population can die. Aquaponics has been developed as an alternative for water purification and conservation (Dediu *et al.*, 2011). This technology does not impact water bodies and does not deteriorate soil (Ramírez *et al.*, 2008).

Image by Paul at Pixabay

In the aquaponics system, the organic waste dissolved and suspended in the tank water is used as a source of nutrients for the development of edible and/or commercial plants (García-Ulloa *et al.*, 2005). In the first stage, water passes through a clarification tank where the suspended solids are precipitated; the water is subsequently filtered (using sand, gravel, plastic or other materials) to capture fine solids. After that, water passes through the mineralization station where (aerobic) bacteria transform the dissolved material. As the penultimate stage, dissolved gases (carbon dioxide, methane and hydrogen sulfide) are released. Finally, water flows into the system (where the plants, floating raft or inert bed are located) and returns to the fish tank (Van Gorder, 2003; Ramírez *et al.*, 2008; Dediu *et al.*, 2012).

A biofilter is a place where bacteria are provided with large areas to colonize and perform NH_3 a NO_3^- transformation, which is essential for water quality parameters in aquaponics systems. *Oreochromis niloticus* and *Lactuca sativa* have been produced in spaces where solids were previously removed; the plants work as an additional biofilter (Al-Hafedh *et al.*, 2008) for the removal of nitrogenous species. Moreover, Ramírez *et al.* (2009) used a sand and gravel filter in a *Carassius auratus* - *Lactuca sativa* system, obtaining NH_3, NO_2^-, and NO_3^- concentrations that ranged from 0.1 to 6.7 mg L^{-1}, from 0.1 to 6.0 mg L^{-1}, and from 0 to 80 mg L^{-1}, respectively. Danaher *et al.* (2013) compared water quality parameters, using a cylinder-conical clarifier and a vortex separator for the primary treatment of solid waste in a *O. niloticus* - *Ipomoea aquatica* culture. However, they did no find significant differences in TAN (total ammonia nitrogen), NO_2^- and NO_3^- concentrations.

In aquaponics, all the filtration components are important (clarifier, mineralizer, and degasser), but their operation requires equipment and space. An alternative to the situation is to replace the three above-mentioned elements with a single biofilter. Therefore, the objective of this study was to determine the water quality of an aquaponics system through the integration of a biofilter in the process.

MATERIALS AND METHODS

Aquaponics system

The structure of the aquaponics system consisted of a fish tank, a biofilter, a NFT (Nutrient Film Technique) system as a grow media for plants, and a water pump to recirculate the water. A Frame Pool™ semi-rigid pool (399 cm long × 211 cm wide × 66 cm high) was used as an aquaculture tank. It operated at 90% of its total capacity (3,300 L). The upward flow biofilter (100.00 cm long × 15.24 cm diameter) was built with polyvinyl chloride (PVC) tubing. Sponge gourd (*Luffa cylindrica*) cubes with 3.0 cm edges, previously washed, were used as the inert substrate of the biofilter. The filter was connected in series to the NFT system with five pre-drilled PVC tubes (300 cm long × 15.24 cm diameter). Nineteen holes for plant growth were drilled in each tube. The resulting total holes (95) were filled with tezontle and were used to hold 300-ml containers. Wormseed (*Chenopodium ambrosioides*) seedlings were then planted in the perforations. Water recirculation was performed with a 3,500 L d^{-1} water pump that connected the fish tank and the biofilter.

Hydraulic conditions and fish and plant culture

In two 90-day trials, 140 L h^{-1} of water were recirculated in the aquaponics system. Once the recirculation system was structured, an initial sampling of the water, fish and plants was performed. Subsequently, the physicochemical water parameters and biometry of fish and plants were determined every 15 days. In the aquaculture component, 148 tilapia (*Oreochromis niloticus*) fingerlings —weighing 14.31 g and measuring an average of 7.26 cm long— were placed. Fish were fed with a 32% protein commercial diet (Silver Cup "El Pedregal"). The daily ration was estimated at 5% of live weight day^{-1}. Food was administered in two portions, at 8:00 a.m. and 4:00 p.m. Wormseeds (*Chenopodium ambrosioides*) were germinated in an Andosol and, 15 days after germination, seedlings were transplanted to the containers (after the roots were washed with running water) and randomly placed in the NFT system. Fish and plants culture was performed for 91 days with two replicates.

Fish length, weight, and survival

The length and weight of each individual fish were determined every 15 days. Length was determined from the head to the caudal peduncle with an ichthyometer and the weight was measured with a Rhino® precision analytical balance. Fish survival was recorded daily by visual inspection of the tank. The weight and length values were used to calculate the weight gain and length of the tilapia The feed conversion was obtained by dividing the total food supplied by the total biomass produced.

Height, weight, chlorophyll content, and survival of plants

The height of the plants was determined using a metric rule (cm) at the time of pruning and harvesting. The plants were pruned 45 days after sowing (dds) and the harvest was carried out at 90 dds. An effort was made to leave 5-cm high stolons after the pruning. During the harvest stage, plant height was measured from the base to the apex. The fresh weight of plants was established with a Rhino® precision analytical balance. Before the plants were pruned and harvested, their nutritional status was determined by reading the SPAD units with a Konica Minolta® SPAD 502 portable meter.

Physicochemical parameters of water

A Hanna® HI 9829 multiparameter meter was used to determine the physicochemical parameters of water: temperature (°C), pH, dissolved oxygen (mg L^{-1}), and electrical conductivity ($\mu S\ m^{-1}$). Ammonium $\left(NH_4^+\right)$, nitrite $\left(NH_2^-\right)$ and nitrate $\left(NH_3^-\right)$ were determined with a Hach® DR/890 portable colorimeter. Every 7 days, all the parameters were determined in the tank, in the biofilter outlet, and in the outlet of every plant growth device.

Statistical analysis of the data

The data generated in this work were subjected to an analysis of variance (ANOVA) and a comparison of means (Tukey's test, $p<0.05$) with the professional version of the InfoStat software (2011).

RESULTS AND DISCUSSION

Physicochemical parameters of water

Table 1 shows the mean values of the water physicochemical parameters used in the aquaponics system —taken from seven sampling points, during 13 weeks of processing and two productive cycles. The variations in the mean temperature (T), pH, and electrical conductivity (EC) values were not significant. However, variations of DO (1.8-2.9), $N\text{-}NH_4^+$ (0.2-2.0), $N\text{-}NO_2^-$ (0.7-1.4) y $N\text{-}NO_3^-$ (10.4-17.2) values (measures in mg L^{-1}) showed a significant difference.

From the perspective of the sampling points, the interior of the tank, the biofilter outlet, and the point of return to the tank (outlet of the plant growth system), only the DO, NH_4^+ and NO_3^- variables showed significant changes (Table 2). Accordingly, DO increased 21%, while NH_4^+ and NO_3^- decreased 35% and 24%, respectively.

Table 1. Mean temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), NH_4^+, NO_2^- and NO_3^- values of aquaculture water. The value of each of the variables is the mean of the seven sampling points and two crop cycles.

Week	T (°C)	pH	DO (mg L^{-1})	EC (μS cm^{-1})	$N\text{-}NH_4^+$ (mg L^{-1})	$N\text{-}NO_2^-$ (mg L^{-1})	$N\text{-}NO_3^-$ (mg L^{-1})
1	28.5	7.6	2.6 bc	655.6	1.0 cd	1.1 b	14.1 b
2	28.4	7.4	1.9 ef	629.6	0.9 de	0.8 c	17.2 a
3	28.2	6.9	2.4 c	698.5	0.2 f	1.1 b	12.1 cd
4	28.1	7.6	2.8 ab	686.3	1.7 ab	0.7 c	14.5 b
5	27.9	7.3	1.8 f	526.7	0.9 de	1.4 a	10.7 d
6	27.7	7.2	2.6 bc	644.6	1.3 bc	1.2 ab	14.3 b
7	27.6	7.6	2.7 ab	672.0	1.5bc	1.2 ab	13.0 bc
8	27.4	7.3	2.2 cd	644.6	1.0 cd	1.1 b	10.4 d
9	27.3	7.1	2.3 cd	640.2	1.3 bc	1.2 ab	10.7 d
10	27.1	7.6	2.1 cde	627.2	2.0 a	1.1 b	10.8 d
11	27.0	7.4	2.9 a	599.2	1.1 cd	1.2 ab	12.9 bc
12	28.7	7.5	2.6 bc	571.0	1.7 ab	1.1 b	11.5 cd
13	28.8	7.6	2.4 c	596.3	1.0 cd	1.1 b	11.0 d

Values with dissimilar letters were statistically different (Tukey's test, α<0.05).

Table 2. Mean temperature, pH, electrical conductivity (EC), dissolved oxygen (DO), NH_4^+, NO_2^- and NO_3^- value of water within the tank, at the biofilter outlet, and in the point of return of the tank of the aquaponics system. The value of each of the variables is the mean of 13 weeks of processing and two crop cycles.

Site	T (°C)	pH	DO (mg L^{-1})	EC (μS cm^{-1})	$N\text{-}NH_4^+$ (mg L^{-1})	$N\text{-}NO_2^-$ (mg L^{-1})	$N\text{-}NO_3^-$ (mg L^{-1})
Bosom of the pond	27.8	7.2	2.4 b	625.6	2.0 a	1.2	14.5 a
Biofilter outlet	27.8	7.1	2.6 ab	629.9	1.9 a	1.2	13.5 b
Retum of tank	27.7	7.3	2.9 a	687.3	1.3 b	1.1	11 c

Values with dissimilar letters were statistically different (Tukey's test, α<0.05).

In the aquaponics system studied, water quality parameters fall within the values required by the biological components of the system; these results match the findings of Somerville *et al.* (2014). In this respect, DO concentration within the tank seems limited; however, it has been reported that tilapia can develop between 2 and 3 mg L^{-1} (Somerville *et al.*, 2014). pH was maintained within the 6.9-7.6 range, which is suitable for the development of fish and plants (Tyson *et al.*, 2004). Likewise, water temperature was within the appropriate range for the fish species, as well as for plants and nitrifying bacteria (Somerville *et al.*, 2014); meanwhile, the EC fluctuated between 0.53 and 0.70 dS m^{-1} (*i.e.*, a low ion concentration in water).

Using the data reported by Emerson *et al.* (1975) for the $NH_3 \Leftrightarrow NH_4^+$ equilibrium in an aqueous solution, the average ammonia concentration (NH_3, a gas which is toxic to fish) was <0.022 mg L^{-1}, under the pH and temperature conditions of the tank. This concentration is 91 times lower than the level required to detect symptoms of fish poisoning (Somerville *et al.*, 2014); therefore, TAN did not exceed the 3 mg L^{-1} recommended by Timmons *et al.* (2002).

The passage through the biofilter reduced the NH_4^+ and NO_3^- content of the tank water by 5.0% and 6.9%, respectively; meanwhile, the passage of the same water through the culture system decreased their content by 31.6% and 18.5%, respectively. The efficiency with which the biofilter-plant system in culture removed NH_4^+ and NO_3 was 35.0% and 24.1%, respectively. The equivalent amount of TAN removed by the system under study was similar to that reported by Mulay and Reddy (2021) for a system with basil. However, the NH_4^+ and NO_3 removal was 2.5 and 3.6 times lower and 2.3 and 3.3 times lower for the water spinach (*Ipomoea aquatica*) system and for the mustard (*Brassica juncea*) system, respectively (Enduta *et al.*, 2011).

Growth of *Oreochromis niloticus* and *Chenopodium ambrosioides*

The fish growth (length- and weight-wise) was quantified at the end of the cycles. In this period, fish grew, on average, 0.05 cm day^{-1} and gained 0.73 g day^{-1} (Figure 1). The average production of fish biomass was 9.67 kg. The amount of food supplied during the cycle was 23.16 kg. Therefore, the feed conversion ratio (FCR) was 2.38 —although the

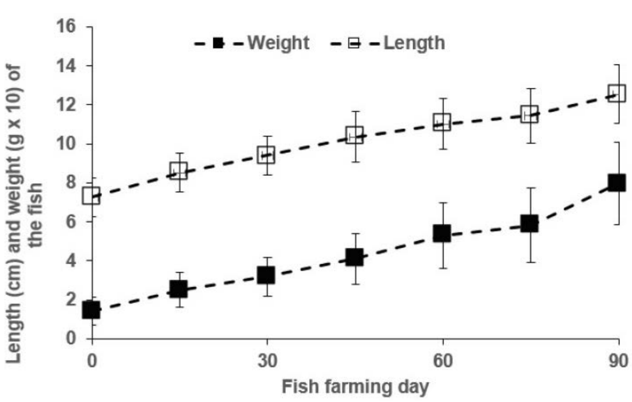

Figure 1. Weight and length gain dynamics of *Oreochromis niloticus*, cultivated for 91 days in an aquaponics system.

FCR is considered acceptable below 2 (Craig and Helfrich, 2009)— and the fish survival was 100%.

The rate of growth and weight gain of wormseed (*Chenopodium ambrosioides*) plants during the 90-day cycle was 0.68 cm day^{-1} and 0.085 g day^{-1}, respectively. A total production of fresh matter of 725.8 g was obtained. The SPAD readings remained unchanged throughout the cycle (33.9 units), which means that the nitrogen concentration in the system is enough for the *C. ambrosoides* production.

CONCLUSIONS

After 91 days of work, the water quality parameters of the tilapia (*Oreochromis niloticus*)-wormseed (*Chenopodium ambrosioides*) aquaponics system —interconnected through a biofilter with a sponge gourd (*Luffa cylindrica*)— were within the recommendations for the said system.

During the time that the work lasted, no symptoms of toxicity or dead fish were recorded. Consequently, the aquaponics system under study had the capacity to maintain ammonia nitrogen below the toxicity concentration.

No mineral deficiency symptoms were observed in wormseed (*Chenopodium ambrosioides*). This result was confirmed by the SPAD level.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Morphological and molecular characterization of *Podosphaera xanthii*, causal agent of powdery mildew in husk tomato and watermelon

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ABSTRACT

Objective: To determine the causal agent of powdery mildew in husk tomato and watermelon, as well as its morphological and molecular characterization.

Design/methodology/approach: Leaves with powdery mildew symptoms were collected from husk tomato (*Physalis ixocarpa*) and watermelon (*Citrullus lanatus*) in Iguala, Guerrero, Mexico in 2018. From two isolates (Phyxa1 and Phyxa2) of husk tomato and two of watermelon (Citrus1 and Citrus2), the morphological characterization was carried out by assembling morphological structures and visualizing them under an optical microscope. For molecular characterization, the ITS region was amplified with the use of primers ITS1 and ITS4, PCR was performed and the products obtained were sequenced in the company Macrogen®. A phylogenetic analysis was performed with the resulting sequences and they were compared with other sequences available in GenBank.

Results: It was determined that there is morphological and genetic variability between isolates from husk tomato and watermelon. The largest sizes of conidiophores and conidia were from Phyxa1 and Phyxa2 isolates, the smallest sizes were found in Citrus1 and Citrus2. The isolates presented a tendency to group according to the host, the Phyxa1 and Phyxa2 isolates were associated with Solanacea isolates, while the Citrus1 and Citrus2 isolates were grouped with isolates of the Cucurbitaceae family.

Findings/conclusions: *Podosphaera xanthii* was shown to be the agent associated with powdery mildew in husk tomato and watermelon. The morphological and genetic variability of *P. xanthii* was determined, which was associated with the host of origin.

Keywords: Fungal diseases, *Physalis ixocarpa*, *Citrullus lanatus*.

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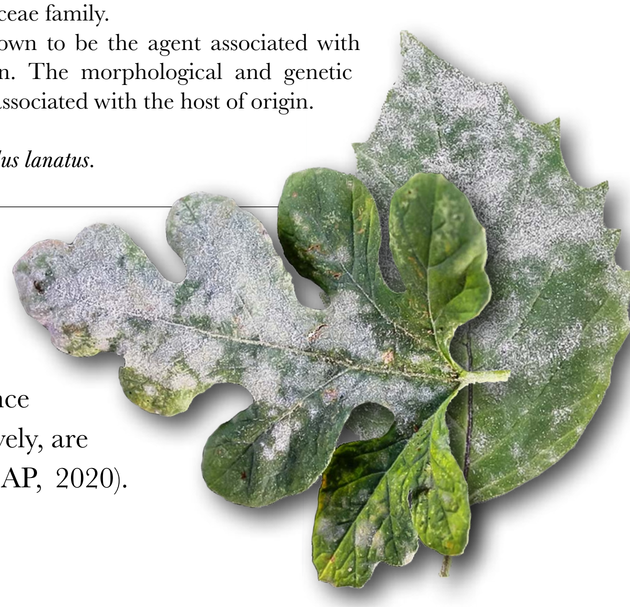
INTRODUCTION

In Mexico, the crops of husk tomato (*Physalis* spp.) and watermelon (*Citrullus lanatus*) are of economic importance, since surfaces of 40,116 and 39,735 ha, respectively, are destined to their cultivation annually (SIAP, 2020).



However, these crops are susceptible to the disease known as powdery mildew. Globally, *Podosphaera xanthii* has been reported as an agent associated to powdery mildew in the crops of husk tomato and watermelon (Kousik *et al*., 2011; Meeboon *et al*., 2016). The most common species of powdery mildew in cucurbits are *Podosphaera xanthii* and *Golovinomyces cichoracearum* (Leão *et al*., 2019). In Mexico, *P. xanthii* has been reported in Sinaloa as the causal agent of powdery mildew in *Physalis ixocarpa* and *Citrullus lanatus* (Félix-Gastélum *et al*., 2005; Félix-Gastélum *et al*., 2007).

On the other hand, grouping fungi taxa by molecular phylogeny has shown a general relationship with grouping by infectivity, which suggests that the separation of niches caused by specialization of the host unleashes the genetic divergence of these fungi (Hirata *et al*., 2000). In this sense, Takamatzu *et al*. (2013) performed a phylogenetic analysis through the sequence analysis of ITS and 28S regions of the rDNA of species of powdery mildew of the genus *Golovinomyces* and determined a high evolutionary relationship with their host plants. Other studies have shown the usefulness of the nuclear sequencing of ribosomal DNA such as the ITS and 28S regions to determine the phylogenetic relationships of species of powdery mildew (Saenz and Taylor, 1999; Bradshaw *et al*., 2020). Recently, in the tropical environmental conditions of Iguala de la Independencia, Guerrero, considerable damage was detected in husk tomato and watermelon crops, with impact of 100% in that municipality; based on a literature review, the species of powdery mildew in husk tomato and watermelon are unknown.

Therefore, this study had the objective of determining the causal agent(s) of powdery mildew in husk tomato and watermelon, as well as their morphological and molecular characterization.

MATERIALS AND METHODS

Collection of plant material

In production family gardens of Iguala, Guerrero, leaves were collected in January 2018 with symptoms of powdery mildew in husk tomato (*Physalis ixocarpa*) and watermelon (*Citrullus lanatus*) plants during the phenological stage of flowering and fruit development, finding an impact of 100%.

Morphological characterization

Samples of husk tomato and watermelon leaves infected with powdery mildew were collected and placed in plastic bags. With the use of a binocular stereoscopic microscope, small portions of mycelium were transferred to a slide and tinted with cotton lactophenol blue. An optical microscope was used (Nikon®) for the morphological characterization. From each isolate/host, 100 conidiophores and conidia were measured with an AmScope® chamber. Microphotographs of conidia and conidiophores were captured.

Molecular characterization

Based on two isolates (Phyxa1 and Phyxa2) of leaves with symptoms of powdery mildew in husk tomato and two in watermelon (Citrus1 and Citrus2), mycelium was scraped directly from the symptomatic tissue of each isolate with the help of a stereoscopic microscope and was deposited in sterile Eppendorf tubes of 1.5 ml. The DNA was extracted through the CTAB method (Doyle and Doyle, 1990) and the concentration was determined in a Nanodrop® 2000 spectrophotometer (Thermo Scientific, MA, USA). The region of internal transcribed spacer (ITS) of the nuclear ribosomal region of DNA was amplified for all the isolates with the use of universal primers ITS1 and ITS4 (White *et al.*, 1990). The PCR mixture consisted of: 2 μL buffer (1 X), 0.6 μL MgCl2 (2.0 μM), 0.2 μL dNTP's (0.2 mM), Primer (10 μM) forward 0.6 μL and reverse 0.6 μL, 0.1 μL Taq DNA polymerase (0.5 U) (Promega®), and 20 ng of the DNA extracted from each isolate. The mixture was adjusted with ultra-pure sterile water at a final volume of 10 μL. The PCR amplification was carried out in a thermocycler (Techne-TC-512®), for which the following amplification program was used: initial denaturalization of 5 min at 94 °C, followed by 35 cycles that consisted of 40 s of denaturalization at 94 °C, aligning of 1 min at 62 °C, and final extension of 1.5 min at 72 °C with a final extension of 5 min at 72 °C. The amplified PCR products were purified with the Wizard®SV Gel kit and PCR Clean-Up System (Promega®) following the instructions from the manufacturer. The products were verified in agarose gels at 1.5% in a horizontal electrophoresis system during 85 min at 45 V (Thermo Scientific™ Owl™ Horizontal Gel Electrophoresis Systems) and visualized in a UV light trans illuminator (First Light® Illuminator), and sequenced at the Biotechnology Institute in UNAM. The sequences were edited and assembled with the DNABaser® ver 4.0 software. The sequences obtained in this study were deposited in GenBank.

Phylogenetic analysis

A phylogenetic analysis of the ITS region was conducted with the sequences of the isolates obtained from husk tomato (Phyxa1 and Phyxa2) and watermelon (Citrus1 and Citrus2). The sequences were compared with other isolates available in GenBank of *Podosphaera xanthii*, and related species (*P. macrospora*, *P. leucotricha* and *P. macularis*), and *Cystotheca lanestri* was used as organism outside the group. The sequences were homologated at 489 pb. The phylogenetic analysis was carried out with the Maximum Likelihood method, and the Tamura-Nei model (Tamura and Nei, 1993), for which 1000 bootstrap replicates were used, the analyses were conducted with MEGA® ver. X. (Kumar *et al.*, 2018).

RESULTS AND DISCUSSION

In this study *Podosphaera xanthii* was identified as the agent associated to powdery mildew in husk tomato and watermelon in Iguala, Guerrero, Mexico. The symptoms present in leaves were white powdery colonies, with circular or irregular shape, which invaded 100% of the leaf surface, and caused necrosis and premature senescence (Figure 1A and 1B).

Morphological characterization

Morphological variability was detected in the isolates from husk tomato and watermelon, which was influenced by the host of origin (Table 1).

Podosphaera xanthii isolated from husk tomato presented flexuous, ramified and septate hyphae. The conidiophores were straight to slightly curved, non-ramified and cylindrical and measured on average 181.0×12.7 and 178.1×12.2 μm (length×width), for the

Figure 1. Symptoms of powdery mildew; A=Powdery mildew in husk tomato (*Phyxalis ixocarpa*). B=Powdery mildew in watermelon (*Citrullus lanatus*). C=Conidia and conidiophores of *Podosphaera xanthii* isolated from husk tomato. D=Conidia and conidiophores of *P. xanthii* isolated from watermelon. Bar=20 μm.

isolates Phyxa1 and Phyxa2, respectively. They presented doliform and ellipsoid conidia, and measured 37.3×18.2 and 36.6×18.4 μm (length×width), for the isolates Phyxa1 and Phyxa2, respectively. The length×width rate was 2.1 and 2.0 for Phyxa1 and Phyxa2, respectively.

P. xanthii isolate from watermelon produced flexuous, ramified and septate hyphae. The conidiophores were straigth to slightly curve, non-ramified and cylindrical, measures 158.0×11.7 and 157.6×11.6 μm (length×width) for Citrus1 and Citrus2, respectively. The conidia were doliform and ellipsoid, and measured 29.69×16.0 and 30.7×15.9 μm (length×width) for Citrus1 and Citrus2, respectively, and an average length×width rate of 1.9 (Citrus1) and 2.0 (Citrus2). The isolates Phyxa1 and Phyxa2 presented statistically the largest size of conidiophores and conidia when compared with the isolates Citrus1 and Citrus2 (Table 1).

Based on the morphological characteristics, the fungi identified from leaves of husk tomato and watermelon correspond to *P. xanthii* (Braun and Cook, 2012). The conidia and conodiophores of larger size were observed in the isolates Phyxa1 and Phyxa2 from husk tomato; these same isolates presented the highest length/width rate of conidia. The isolates analyzed from watermelon presented the smaller sizes regarding the characteristics

Table 1. Morphological characteristics of *P. xanthii* isolated from husk tomato and watermelon (*Citrullus lanatus*), from Iguala, Guerrero, Mexico.

Isolation	Conidiophores						Conidia						LWR
	Length			Width			Length			Width			
	Min[y]	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	
Phyxa1	92.7	274.3	181.0 a[z]	9.8	15.4	12.7a	28.5	46.7	37.3 a	13.7	24.5	18.2 a	2.1
Phyxa2	122.7	274	178.1 a	11	15.09	12.8a	26.2	44.9	36.6 a	12.2	23.8	18.4 a	2.0
Citrus1	113.1	212.6	158.0b	9.4	15.3	11.7b	22.4	35.1	29.7 b	13.3	19.0	16.0b	1.9
Citrus2	120.8	210.2	157.6 b	10.1	13.8	11.6 b	25.2	35.5	30.7 b	13.1	20.8	15.9b	2.0

[y]=Min=minimum size of conidiophores or conidia in μm. Max=maximum size of conidiophores or conidia in μm. Avg=average in μm. LWR=Length/width rate.

[z]=Means with the same letter in the same column are not significantly different (P=0.01) based on Duncan's multiple range test.

aforementioned (Table 1). The results suggest that there is morphological variation of *P. xanthii* based on their host of origin. In a study carried out in Taiwan, Yeh *et al.* (2021) reported notable morphological variability of *P. xanthii* in the anamorph stage, which was influenced according to the host where it was isolated; similar results were determined in this study.

Molecular characterization

The sequences obtained from amplification of rDNA of the ITS region from the isolates of husk tomato and watermelon were deposited with the following numbers of access: Phyxa1 (GenBank: MH238506), Phyxa2 (MH238507), Citrus1 (MH238508) and Citrus2 (MH238509).

The phylogenetic analysis demonstrated that there is genetic diversity of *P. xanthii* at the level of amplified region (ITS), according to the host from which it was isolated; for their part, the isolates obtained from husk tomato (Phyxa1 and Phyxa2) presented a tendency to group with isolates from *P. xanthii* isolated from *Petunia*×*hybrida* (Solanaceae) from the United States of America. Meanwhile, the isolates Citrus1 and Citrus2 grouped with diverse species of Cucurbitaceae and other families, for example *Sechium edule*, *Cucurbita moschata*, *Cucumus sativus*, *Cucumis melo*, *Momordica charantia*, among others (Figure 1). In this regard, in USA Xiang *et al.* (2020) evaluated the genetic variability of *P. xanthii* isolated from *Cucurbita* spp. and *Lagenaria siseraria* through the amplification of the ITS region, this variation was strongly influenced by the place of collection of isolates and the host. In addition, in Italy, through a RAPD analysis, Miazzi *et al.* (2011) reported high genetic variability of *P. xanthii* isolated from *Cucurbita* spp., while in this study only the sexual phase of *P. xanthii* was detected; however, the sexual phase has been reported in Sinaloa, Mexico,

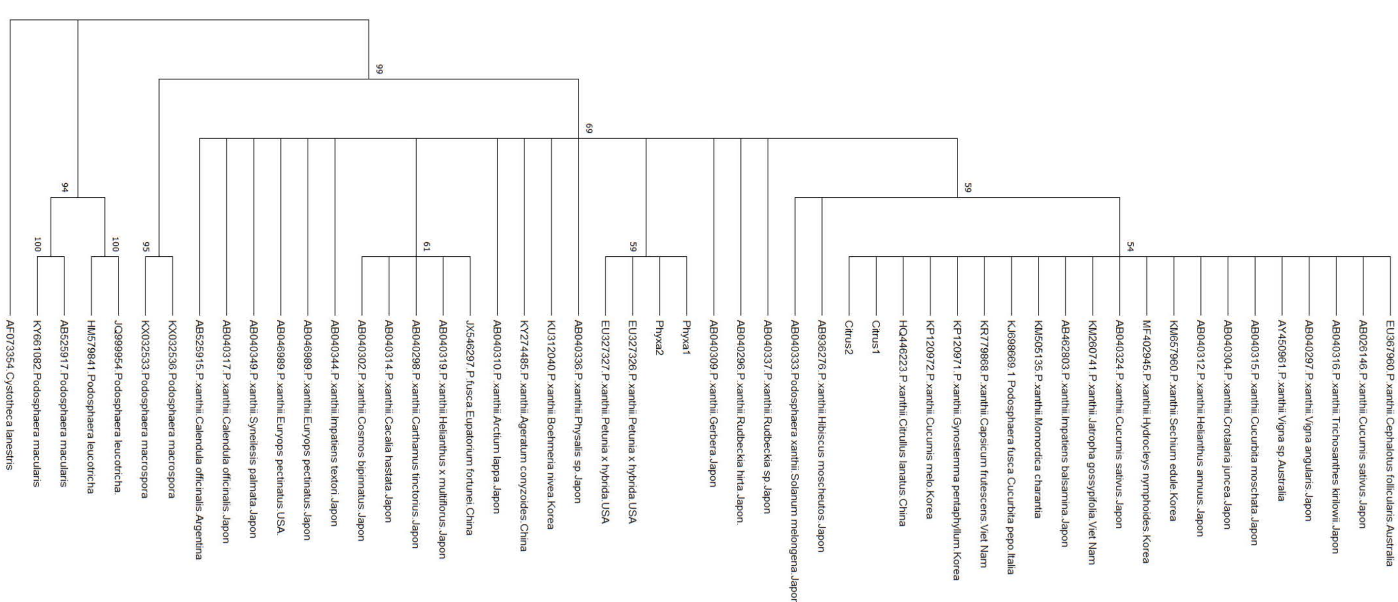


Figure 2. Phylogenetic tree based on sequences of the ITS region of *P. xanthii* isolates Phyxa1, Phyxa2, Citrus1 and Citrus2, and their relationship with other isolates. *Cystotheca lanestri* was used as organism outside the group. The phylogenetic tree was constructed in MEGA X, using the Maximum Likelihood method and the Tamura-Nei model with 1,000 bootstraps.

in hosts of squash and cucumber (Félix-Gastélum *et al.*, 2005). Considering the lack of sexual reproduction, the genetic variability mechanism of *P. xanthii* could happen through the presence of parasexuality in the hyphae, and in addition for powdery mildew its control is generally carried out through the application of fungicides, which is why the mutation to develop resistance to fungicides is another possibility for developing genetic variability (Vielba-Fernández *et al.*, 2020; Xiang *et al.*, 2020). It is necessary to perform other research studies to generate greater knowledge on the biology of *P. xanthii* in Guerrero, Mexico, in order to contribute useful information for the management of powdery mildew in husk tomato, watermelon and other crops of importance for the diet that are susceptible to this pathogen.

CONCLUSIONS

This study shows that *P. xanthii* is the agent associated with powdery mildew in husk tomato and watermelon. The morphological and genetic variability of *P. xanthii* was determined, which was associated to the host of origin. The conidiophores and conidia were of larger size in husk tomato, while the smaller sizes were determined in watermelon. The phylogenetic analysis demonstrated a tendency of the isolates from husk tomato to be associated to isolates from Solanacea, while the isolates from watermelon to be grouped with isolates from Cucurbitaceae.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Maize silage and maize stubble, strategy for cattle feed in dry seasons

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ABSTRACT

The objective was to estimate the production costs and profitability of maize silage and maize stubble as a feeding strategy for cattle in dry seasons in the southern region of Estado de México.

Methodology: 30 semi-structured surveys with non-probabilistic sampling were applied during the spring/summer 2020 cycle. Socioeconomic variables, the profitability threshold and the benefit/cost ratio were analyzed. The production units were characterized as small, the average age of producers did not exceed 45 years and nine years dedicated to production.

Results: The average production of maize silage and maize stubble was 28 and 7.20 tons with a cost of 1,278.40 and 3,587.23 pesos per ton.

Conclusions: The benefit/cost ratio in maize silage and stubble was 0.46 and 0.16. The conclusion is that both activities were profitable with a predominance of maize silage.

Keywords: economic analysis, production costs, maize silage, maize stubble, benefit/cost ratio.

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INTRODUCTION

Among cereal production in the world, maize is the one with highest production. Annually, it has a volume of 850 million tons in grain cultivated on a surface of 162 million hectares, with an average production of 5.2 tons per hectare (t/ha). Mexico is the second maize importer and it is supplied from the United States and Argentina. Germany and France are the main producers of fodder maize (Cruz, 2021). In Mexico around 70 million tons of agricultural residues are produced, of which the stubble of maize, sorghum and wheat straw represents 58, 12 and 15%, respectively. There are methods to treat the fibrous fodders that allow increasing the consumption, feed digestibility, and animal production (Fuentes *et al.*, 2001). Maize in Mexico is used for human and animal consumption. In the case of animal consumption, it is used as fresh fodder, silage or stubble, with its use mainly during the dry season (Luna *et al.*, 2013).

Image by Markus Distelrath at Pixabay

The use of maize stubble is common as feed for ruminants, although it has low nutritional value, low digestibility and it is coarse, due to its state of lignification. Interest for the use of agricultural residues in the diet of ruminants has increased their importance in the global scope in recent years, as the availability of grains is reduced. The null competition between monogastric and ruminants over fibrous foods is also important, as well as the ability of ruminants to convert these fibrous materials into useful products for humans (meat, milk, leather, wool, etc.) (Fuentes *et al*., 2001).

Therefore, the objective was to estimate production costs and profitability of maize silage and maize stubble as feed strategy for cattle in dry seasons in a region of southern Estado de México. The main hypothesis assumes that the use of maize silage and maize stubble as dietary strategy for cattle in the dry season reduces production costs, which makes it a strategy that ensures the economic profitability of cattle producers in the study zone.

MATERIALS AND METHODS

To gather the primary information, the decision was made to use semi-structured surveys that were applied through direct interviews to thirty producers of maize silage and maize stubble in the spring/summer 2020 cycle, and information related to the ensilage process in 2020. Non-probabilistic sampling was used, called intentional sampling or judgement sampling, similar to the snowball method (Goodman, 1961; Vogt & Burke Johnson, 2016). This sampling is applied when the statistical sample to be formed is selected in the environment close to the researcher, without there being specific requirements, although trying to sample at least 10% of the total population.

The secondary information came from various official sources: National Institute of Forestry, Agricultural and Livestock Production Research (*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias*, INIFAP), Ministry of Agriculture and Rural Development (*Secretaría de Agricultura y Desarrollo Rural*, SADER), Ministry of the Farmland (*Secretaría del Campo*, SC), National Institute of Statistics and Geography (*Instituto Nacional de Estadística y Geografía*, INEGI), and also information contained in different webpages.

Regarding Rebollar (2011) and Rebollar *et al.* (2022), at the private level the costs were classified into fixed and variable. The first do not depend on the production volume, and it must be assumed that even when there is no production, they remain both in the short and in the long term and represent the proceeds (negative) in the absence of production. The second represent the real disbursements linked to the payment for purchase of variable inputs; they happen when there is production, depend on the amount produced, and change when the volume produced changes.

In fixed costs, the useful life of the fixed or immobilized asset was emphasized, as well as its purchase price, years of utility, divided into months of the year and multiplied by the amount of product generated. For the variable expenditures, the cost of the input was multiplied by the amount used. All of this is for each producer surveyed, and then the total cost per producer, its variable, fixed and mean total cost were obtained. Subsequently, an average of the cost was estimated when considering the total of producers surveyed.

Therefore, the total cost per activity (Equation 1).

$$(TC) = VC + FC = PxX + FC \quad (1)$$

where *Px* was the price of the variable input, and *X* the amount used. The total income (*TI*) per sale was obtained when multiplying the amount of final product by the average price current in the local market. Thus, the *TI*=*PyY*, where *Py* was the price per ton of product obtained and *Y* the amount of product that was obtained, for the sale.

In addition, the profit from the process was calculated as the arithmetic difference between the *TI* minus the *TC*; that is (Equation 2):

$$\text{Profit } (P) = TI - TC = PyY - (PxX + FC) \quad (2)$$

Therefore, if the *P* is higher than zero, it will be evidence of the profitability of the process; otherwise, there will be economic loss in the production. Likewise, the value of the profitability indicator known as Benefit/Cost Ratio (*B*/*C R*) was determined as the quotient of dividing the *TI* from sales by the *TC* of production; that is (Equation 3):

$$B / C\ R = (\$TI) / (\$TC) \quad (3)$$

A B/*C R* quotient higher than 1.0 is evidence of profitability per peso invested in the activity. An additional indicator of the activity was the point known as profitability threshold (Rebollar Rebollar, 2011), called minimum economic productive capacity (MEPC), known as the equilibrium point (EP). The expression to be used was (Equation 4):

$$EP(Q) = \frac{\text{Total fixed cost}}{\text{Sale price} - \text{mean variable cost}} \quad (4)$$

and:

$$EP(\text{pesos}) = \frac{\text{Total fixed cost}}{\frac{\text{Sale price minus mean variable cost}}{\text{Sale price}}}$$

This indicator helped to deduce whether the activity was profitable or not, based on the methodology that was used in the study (Rebollar, 2011).

RESULTS AND DISCUSSION

Socioeconomic characteristics

Based on the production system of maize silage and maize stubble, the production units (PUs) were characterized as small-scale, because the surface destined to sowing the crop corresponded to less than one hectare; it ranged between 0.9±0.5 ha for maize silage and 1.2±0.6 ha for maize stubble. The average age of producers did not exceed 45 years (44±8.8, 43.3±14.8) and the number of children per producer was 2.0±1.5 and only

28% of them have four children, who support the activities of the system inconsistently; therefore, it is deduced that the production of maize silage and maize stubble is carried out by producers with basic academic education and with an age range that did not exceed 50 years of age (Table 1).

Analysis of the production system of maize silage and maize stubble

The commercial seeds used were D-Kalb 75-00 with 70% of use for maize silage and 83% for maize stubble, Piooner P4039 with 15 and 8.50% respectively, and the remaining producers leaned towards traditional sowing, when using landrace seeds from previous harvests (Table 2).

In relation to the average surface of land sown devoted to the production of maize silage, it was 0.89 ha, and 1.15 ha for production of maize stubble with an average sowing density of 20 and 15.9 kh/ha and average production of 28 t/ha of maize silage and 7.2 t/ha of maize stubble (Table 3).

Variable costs

For maize silage, the average variable cost was 35,345.3 and 25,268.7 $/ha for maize stubble and corresponded to the totality of the activities carried out in the entire productive process (Table 4).

Table 1. Socioeconomic characteristics in the production of maize stubble and maize silage.

Concept	Maize stubble (value)	Corn silage (value)
Age (years)	43.30±14.80	44.00±8.80
Family integration (children)	1.80±1.30	2.00±1.50
Planted hectares	1.20±0.60	0.90±0.50
Time in activity (years)	11.30±8.00	7.00±3.10
Schooling (years)	10.30±3.80	12.90±4.80

Table 2. Seeds used in maize sowing.

Concept	Maize stubble (value)	Corn silage (value)
Dekalb 7500 seed (%)	83.00	70.00
Pioneer P4039 Seed (%)	8.5.00	15.00
Seed of the previous harvest (%)	8.5.00	15.00
Total	100%	100%

Table 3. Production data of maize silage and maize stubble.

Concept	Maize stubble (value)	Corn silage (value)
Area for corn silage (hectares)	1.15	0.89
Planting density (kilograms/hectare)	15.90	20.00
Production (tonnes)	7.20	28.00

Table 4. Variable production costs in maize silage.

Performed activities	Maize stubble (value)	%	Corn silage (value)	%
Soil preparation	(13 500,00±1 587)	5,50	(16 500±836,50)	5,50
Sowing	(11 300,00±794,10)	4,50	(13 100±668,40)	4,30
Fertilization	(81 045,00±4717,30)	32,70	(108 870±4 465,30)	35,90
Seed used	(11 387,30±525,20)	4,60	(24 295.3±1 027,10)	8.00
herbicide and insecticides	(25 380,00±2359,90)	10,20	(16 995±732)	5,60
Silage Process	(80 185,00±4335,80)	32,40	(99 565±3 165,70)	32,90
Indirect Inputs	(24 620,00±1898,20)	9,90	(23 900.0±946,20)	7,80
Average variable cost	35 345,30	14,20	25 268,70	8,30
Total	247 417,30	100	303 225	100

Fixed costs

The fixed costs reached to produce maize silage averaged $450.4/ha, product of the use of various agricultural tools, while for the case of maize stubble the fixed cost was $559.3/ha.

Total cost (TC)

The total costs reached for production of maize silage were 35,795.7±(11,725.1) $/ha, while the total costs for maize stubble were 25,828.1±10,158.8 $/ha. The mean total cost (MeTC) or cost per producer was $35,795.7±(11,725.1). If average production of silage was 28 tons, then each producer disbursed $1278.4/t.

The mean total cost (MeTC) or cost per producer to produce maize stubble was $25,828.1±10,158.8. If the average production was 7.2 tons, then during the period of analysis the producer paid 3,587.2 $/t produced.

Total income per sale

The total income (TI) is all the money that the enterprise obtained from the sale of products. The total income from the sale of maize silage in relation to the tons produced was (Table 5 and 6):

Table 5. Total income in the production of maize silage.

	Yield (t)	Sale price ($/t)	Total income ($/t)
Average	28.0 (±18,7)	2 000.00 (±360.5)	52 500.00

Table 6. Total income in production of maize stubble.

	Yield (t)	Sale price ($/t)	Total income ($/t)
Average	(7.20±3,2)	(4 233.3±249.8)	(30 013.3±12 364.5)

Income, cost and profit per ton of maize silage. On average, for this activity during the period of analysis, the producers generated positive profits, which reached 16,704.3 \$/t (Table 7). The average B/CR in the production of maize silage was 1.5 and meant that for each peso that the producer paid as total production cost per ton of maize silage, he recovered that peso and obtained a profit of 50 cents. In addition, based on the result of the B/CR the producer could sustain an increase in costs of up to 50% in order not to lose or win; however, when considering the reciprocal of the B/CR, then the average income of the producer, from sale of maize silage, would resist a reduction (caused by the sale price) of up to 33.3% and stay in equilibrium.

As for production of maize silage, the producer paid on average 25,821.1 \$/t, received from the sale of the product 30,013.3 \$/t and earned 4,185.2 \$/t. That is, after paying fixed costs and total variable costs inherent to this activity, the profit was positive and the activity was considered as profitable (Table 8). Producing maize silage in that time of the year had an effect on the total and average B/CR of 1.2, and therefore for each peso of the total cost that the producer disbursed to pay for the activity, he received 20 cents as benefits, and with that, the activity is profitable.

In addition, the producer could face up to 20% of increase in the total production cost and remain in a situation of equilibrium with the income from sales, or else, when taking the reciprocal of this indicator, a reduction of 16.6% in the income in order to be equal to the total production cost of maize silage.

Equilibrium point (EP): Maize silage

The equilibrium point (EP) in pesos was calculated as follows:

$$EP(\text{pesos}) = \frac{\text{total fixed cost}}{\frac{\text{sale price} - \text{mean variable cost}}{\text{Sale price}}} = \frac{450{,}40}{\frac{2000 - 1488{,}17}{2000}} = \frac{450{,}40}{\frac{511{,}83}{2000}} = \$1759{,}5$$

$$EP(Q) = \frac{\text{total fixed cost}}{\text{sale price} - \text{mean variable cost}} = \frac{450{,}40}{2000 - 1488{,}17} = \frac{450{,}40}{511{,}83} = 0{,}9$$

The equilibrium point (EP) or profitability threshold is where total production costs are equal to the total income from sales; the total costs include fixed costs plus variable

Table 8. Income, cost, and profit of maize silage.

	Total income (\$/t)	Total cost (\$/t)	Profit (\$/t)	RB/C
Average	30 013.30	25 828.10	4185.20	1.20

Table 7. Income, cost, and profit of maize silage.

	Total income (\$/t)	Total cost (\$/t)	Profit (\$/t)	RB/C
Average	52 500.00	35 795.70	16 704.30	1.5

costs. Thus, based on the EP, the producer would have to have sold and/or produced 0.87 t of maize silage for the production cost to be equal to the income from sale, situation that makes the system viable, which constitutes a source of income and a form of subsistence.

Equilibrium point (EP): Maize stubble

The equilibrium point (EP) in pesos was calculated as follows:

$$EP(\mathrm{Q}) = \frac{\text{total fixed cost}}{\frac{\text{sale price} - \text{mean variable cost}}{\text{Sale price}}} = \frac{559{,}38}{\frac{4233{,}33 - 3665{,}3}{4233{,}3}} = \frac{559{,}38}{\frac{568{,}03}{4233{,}33}} = 0{,}4$$

$$EP(\mathrm{Q}) = \frac{\text{total fixed cost}}{\text{sale price} - \text{mean variable cost}} = \frac{559{,}38}{4233{,}3 - 3665{,}3} = \frac{559{,}38}{568{,}03} = 0{,}9$$

Under the conditions suggested, these results mean that during the period of analysis, the producer would have to have produced and/or sold 0.9 t of maize silage for the production cost to become equal to the income from sale, situation that makes the system viable, in addition to being a source of income and form of subsistence.

The education of producers was not an obstacle for the production of maize silage, which agrees with the statement by Gutiérrez (2018), where age and schooling as socioeconomic variables linked to maize production did not represent any difference with the activity. However, there were limiting factors for the production such as sufficient availability of land, financial solvency, and machinery available, similar to what was found by Field (2013).

The production costs can also vary from the type of silo used, since, according to Villalobos-Villalobos *et al.* (2015), there are differences between each type of silo, with the one of horizontal pile being the one of lowest elaboration cost; this is the specific reason why producers from the study zone prefer the use of the horizontal silo.

With relation to the utility and importance of maize silage as dietary strategy for animal production, the results found are similar to those reported by Garcés Molina *et al.* (2004) and Gutiérrez (2018) which conclude that the use of maize silage is useful and important for the diet of the herd in addition to being financially profitable (Muñoz *et al.*, 2013).

The yield per hectare of maize stubble (7.2 t/ha) was lower than the one found by Salinas and Gutiérrez (2000) where the authors mention that the production of maize stubble generated by one maize crop (reeds, leaves and cobs), fluctuates between 20 and 35 t/ha, and of stubble only (reeds and leaves) it varies between 16 and 25 t/ha; however, it was higher than the finding by Muñoz *et al.* (2013) in a study for the high valleys in Mexico. Regarding the use of maize stubble as alternative for animal feed, the results obtained agree with those from Asmud and Lars (1983), which argue that promoting the optimal use of maize stubble allows to reduce production costs derived from animal feed. The perception of producers about the utility, importance and ease of use of maize silage plays an important role in the decision for its adoption, which indicates that they are fundamental factors in decision making of the producer, for the adoption or rejection of new innovations in their production unit, as was observed by Martínez-García *et al.* (2016).

CONCLUSIONS

The economic activities of production and use of maize silage and maize stubble, as feeding strategy for animals during the period of study, are sustained under the strategy of substituting the use of commercial feeds for animal diet, and they reduce production costs and improve profitability in agriculture and livestock activities. The economic analysis of production and use of maize silage and maize stubble allows stating that both activities are economically profitable given that the income from sale was higher than the total production cost. The financial activity of maize silage production presented a higher profitability index compared to the use of maize stubble.

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Use of unmanned aerial vehicles (UAVs) for the dasometric analysis of bamboo plantations from the genus *Guadua* spp.

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ABSTRACT
Objective: bamboo is a forest resource that, due to its rapid growth, requires frequent evaluations (monitoring) to define the most appropriate management strategies; however this entails a high cost and a great investment of time. This study presents an analysis of the use of unmanned aerial vehicles (UAVs) to generate information on the crown cover of *Guadua* spp. bamboo strains, and relates it to other of its dasometric parameters.
Methodology: the areas of the bamboo strains were defined based on generated aerial images, where each strain was delimited by differentiating it from its environment, for which four types of thresholds were defined.
Results: the relationship of the crown area with each dasometric parameter suggests that there is a positive trend, where in most cases there was an adequate significance ($P<0.05$): height $R^2=0.67$ ($P=0.0222$); diameter 1.3 m $R^2=0.56$ ($P=0.0367$); culm diameter 0.3 m $R^2=0.57$ ($P=0.0313$); and number of culms $R^2=0.54$ ($P=0.130$).
Conclusions: in this way, the results showed that with the UAV it was possible to determine the coverage area of individual bamboo strains and that some of their dasometric parameters could be estimated based on their allometric relationship.

Keywords: drone, orthoimages, biomass, photogrammetry, allometric equations.

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INTRODUCTION

The specific characteristics of bamboo, such as the quality of its wood and its fast growth (Ruiz-Sánchez, 2019), define it as a promisory species of high ecologic, economic and social value. Because of this, its production has been supported through commercial plantations, where its fast growth must be considered to define the most adequate management strategies, which implies conducting frequent evaluations (height, number of culms, volume, condition) of the growth dynamics (Ruíz-Sánchez *et al.*, 2015). However, these evaluations are limited by the cost and time involved, which is why

Image by Jalyn Bryce at Pixabay

alternatively they can be carried out remotely, through the use of satellite images (Dash *et al.*, 2018). However, they have a low resolution and lose sharpness when there is an intention to work at the level of individual strains. As an alternative, aerial photographs can be generated through the use of unmanned aerial vehicles (UAVs), at specific intervals of time and of high spatial resolution (Surový *et al.*, 2018). This way there can be a historical file of the changes in the plantation with the passing of time (Mohan *et al.*, 2017), with which its structural dynamics can be determined (Tang and Shao, 2015). The use of UAVs is complemented with field evaluations (Panagiotidis *et al.*, 2017), and allows generating information for an intensive management of the plantation. However, there is scarce information regarding bamboo. Therefore, the purpose is to evidence the use of UAVs in bamboo plantations as a practical alternative, in the management of bamboo plantations. This is illustrated with information generated in a commercial bamboo plantation located in the state of Colima, Mexico.

MATERIALS AND METHODS

Study area

An experimental bamboo plantation was evaluated (10,000 m^2), which is located in the state of Colima, Mexico: 18° 57' 56" N, 103° 50' 18" W, at an altitude of 40 m. The climate in this region is warm sub-humid, with mean annual temperature of 26 °C, and mean annual precipitation of 690 mm. The bamboo plantation is two years old and made up by 210 plants of four species of bamboo: *Guadua aculeata* Rupr. ex E. Fourn., *Guadua inermis* Rupr. ex E. Fourn., *Guadua amplexifolia* J. Presl and *Guadua angustifolia* Kunth.

Generation of orthoimage

The aerial images, required to generate the orthoimage, were taken using a multirotor DJI Phantom 4 Pro UAV, with a CMOS sensor of 20M pixels and a RGB chamber. Programming the flight with this device was done through the DJI Go™ Pro software. The frontal superposition between the images was established in 80%, while the lateral superposition was of 70%, for which the Pix4D Capture software was used.

Delimitation of individual strains

Each of the georeferenced bamboo strains was associated with the field measurements: a) number of culms; b) basal diameter (cm), taken at 30 cm of height; c) normal diameter (cm), taken at 1.30 m of height, d) height (m), e) health, and f) survival. To delimit individually each bamboo strain in the orthoimage, firstly, they were differentiated from their environment, for which the thresholds of the following coverages were defined (Li *et al.*, 2020): a) young foliage; b) mature foliage; c) soil; and d) shade. Once the coverages were located, a photographetric segmentation process was conducted (Kaartinen *et al.*, 2012) to define the limits of foliage of each, with which their area could be calculated (m^2).

Allometric equations

With the integration of field information and information that can be generated with the UAV, the study sought to estimate parameters which are difficult to measure with simpler estimation variables (Dong *et al.*, 2020). The corresponding allometric equations were generated, where the area of each strain (obtained with the UAV) was correlated with each of its dasometric parameters (number of culms, basal diameter, normal diameter and height). Finally, an estimation table was generated, where specific area classes are related with the dasometric parameters.

RESULTS AND DISCUSSION

Dasometric characterization

The average number of culms was 16, where the largest population was between 10 and 20 culms (6,000 culms/ha) (Figure 1). These results agree with those obtained for *G. angustifolia*, with densities between 3,000 and 8,000 culms/ha (Londoño, 1998). However, other species of woody bamboo, such as *Bambusa oldhamii* Munro (Castañeda *et al.*, 2005) and *G. angustifolia* (Camargo, 2014), report higher densities (between 5,000 and 11,000 culms/ha approximately), which can be due to various factors, such as their adaptability, growth speed, type of growth, maturity, environmental conditions, etc.

In general, the dasometric characteristics of the strains indicate that it is a heterogeneous plantation. However, in the case of the basal diameter at 30 cm (DC), there is a slight dominance of the category of 2.4 cm, with a range found of 0.28 to 3.43 cm, while for the diameter at 1.30 cm of height (DAP) the class of 2 cm predominates slightly, within a range from 0 to 2.86 cm. This implies that the basal part is slightly thicker and denser than the rest of the stem, which is adequate for construction purposes, as is the case of the wood of *G. angustifolia*, since it tends to be a natural cylindrical element (Sánchez-Medrano *et al.*, 2016). In this regard, Camargo (2014) informed about similar values in the increase in average DAP of 5.1 (±1.7) cm, at seven years since establishment in *G. angustifolia* plantations in Colombia. However, the dimensions in diameter were smaller (up to 50%) than those found in guadales in Colombia (Camargo, 2014); this indicates that the plants have still not reached their maximum growth, as cited by Castañeda-Mendoza *et al.* (2005) and Daquinta *et al.* (2007). In terms of height, two classes dominate (1.5 and 3.5 cm), within a range that goes from 0.72 to 4.9 m. This variation of heights is because the young bamboo strains (1 to 7 years of age) still have not reached the maximum height or their maximum growth potential. Carmargo (2014) mentions that species of the genus *Guadua* reach 25-30 m of height in 5 or 6 months in strains older than 7 years.

Information system of the plantation

From the incorporation of all the information of the bamboo plantation that was taken in the field, a specific geographic information system was structured, where each of the strains was georeferenced in relation to the orthoimage of the bamboo plantation. Therefore, given that the location of each of the strains refers to an exclusive number of registry, this allows managing the information referred to the dynamics of each strain where, for example, the following is known: their location (coordinates), number of culms, height, stem diameter, crown diameter, among other factors, which can be aggregated to the system. Specific data of each strain can also be analyzed through the application, for

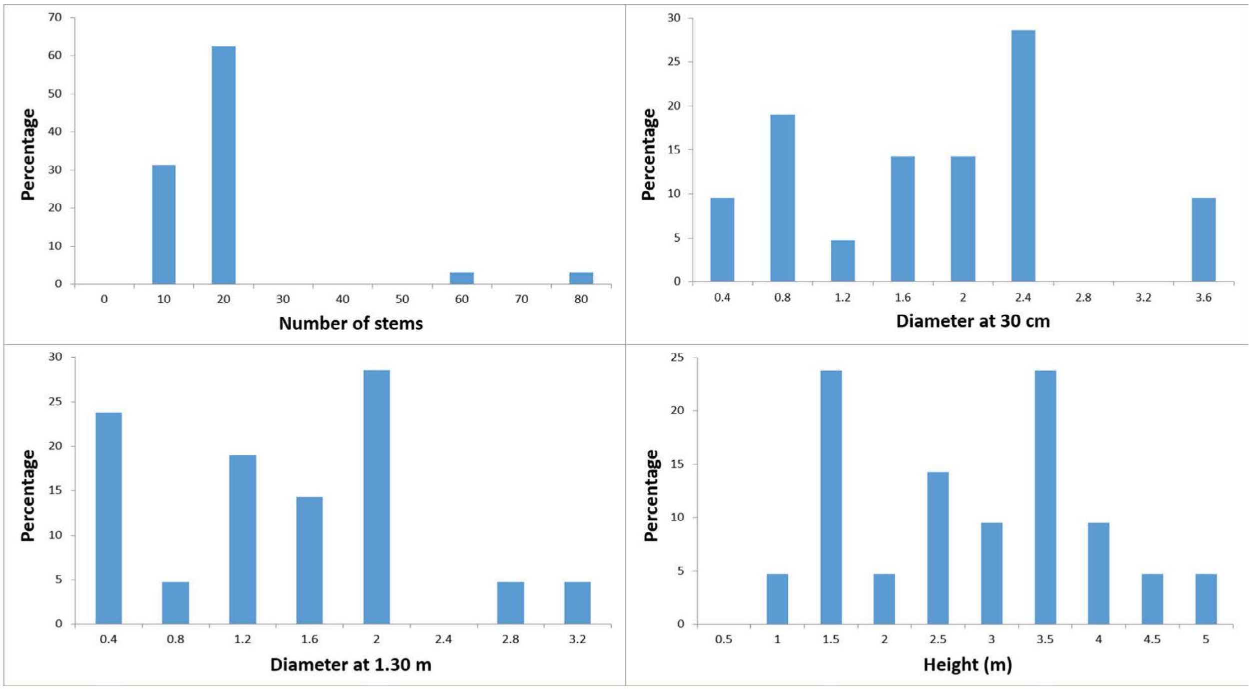

Figure 1. Percentage of dasometric parameters of the bamboo plantation in the Experimental Field in Tecomán, Colima.

example, of a filter per height, which allows graphically locating the place where strains of a certain range of height are found.

Coverage thresholds and delimitation of bamboo strains

To delimit each of the strains, the analysis of the thresholds of the values of the spectral bands of the image allowed differentiating the following coverages (Figure 2): a) shade; b) soil; c) young foliage; and d) mature foliage. Based on this, binary images were generated for each of these coverages (Dong *et al.*, 2020), where, for the case of the bamboo strain, the images corresponding to young foliage and mature foliage were integrated.

Delimitation of bamboo strains

In the photogrammetric segmentation process (Figure 3), it is observed that, in general, the individual outline of the strains is well-defined. Some external portions of young foliage can also be seen, which can be confused with the soil coverage, since the latter considered some zones with herbs. Although it was possible to have better detection in the areas of young bamboo, it was difficult to detect very small bamboo strains. This is important since the ideal composition of a strain in a plantation of *G. angustifolia* must be considered to be 10% of new shoots, 30% of new culms, 60% of mature culms, and to avoid having over-mature culms (Londoño, 1998). This cannot be appreciated only with information from the foliage, as is the case of this study, so better results would be expected in the delimitation of the strains when the plantation has reached maturity (5-7 years).

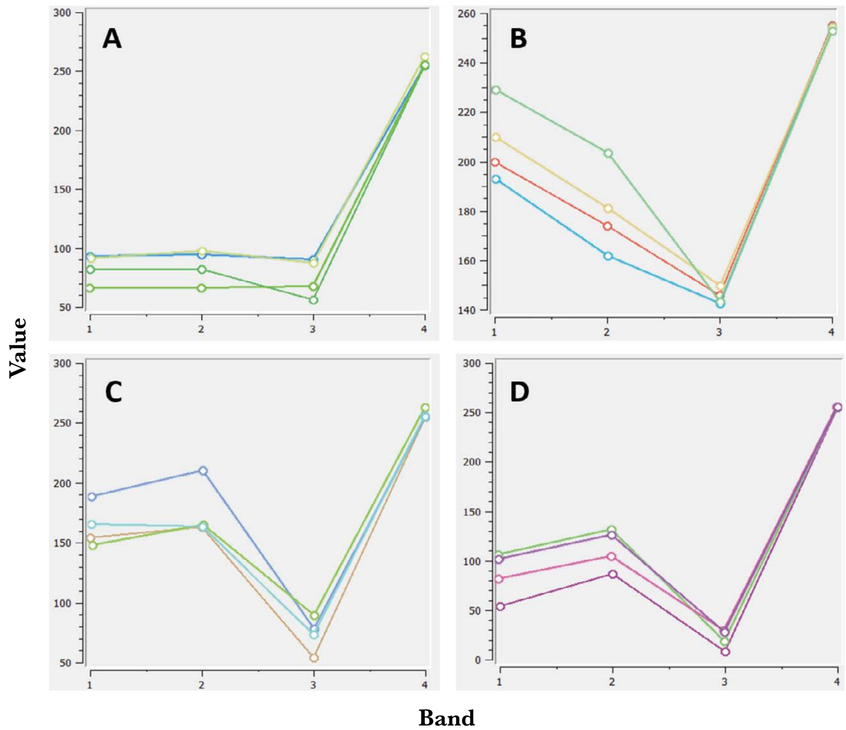

Figure 2. Thresholds of the values of the bands of the orthoimage, corresponding to: A=Shade; B=Soil; C=Young foliage; and D=Mature foliage.

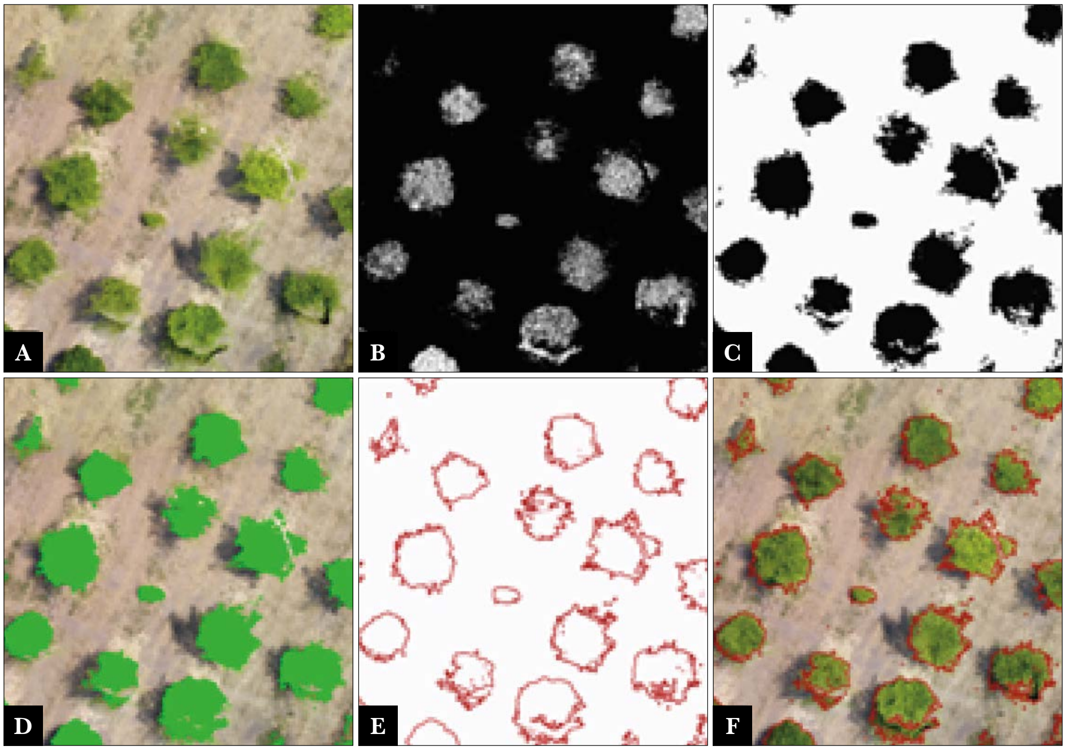

Figure 3. Photogrammetric process to delimit the bamboo strains: A=Orthoimage; B=Image of a band; C=Binary image; D=Area of softened strains; E=Delimitation of bamboo strains; and F=Superposition of polygons on orthoimage.

The remote analysis of the aerial biomass of bamboo is still considered difficult, because there is still a lack of adequate comprehension of the integration mechanisms of the growth characteristics of bamboo forests and data from remote sensors (Yuyun Chen *et al.*, 2019). However, as the bamboo strains grow, the development of the density of the plantation can be monitored through the methodology proposed, in relation to the land coverage and the superposition of the crowns of the strains. Thus, the appropriate moment for the application of clearing in the plantation can be defined, from a silviculture perspective, as: a) sub-dense, when not all the space is used in the best way, leaving many empty spaces; b) over-dense, when there is a large amount of individuals in insufficient space, fostering competition over light and nutrients; and c) optimal density, where all the space of the plantation is used without exposing the trees to competition over resources. Furthermore, when understanding the coverage dynamics of the bamboo strains, through the use of UAVs, growth and yield models can be defined (Letourneau *et al.*, 2011).

Allometric analysis

Figure 4 shows that there is a clear positive trend in the relationship between the area of the bamboo strains and their dasometric parameters, where the relationships of the area with height and diameter at 30 cm were the ones that showed lower variability, grouped more towards the line of defined trends. On the other hand, the relationship with the diameter at 1.30 m was the one that presented the greatest data dispersion.

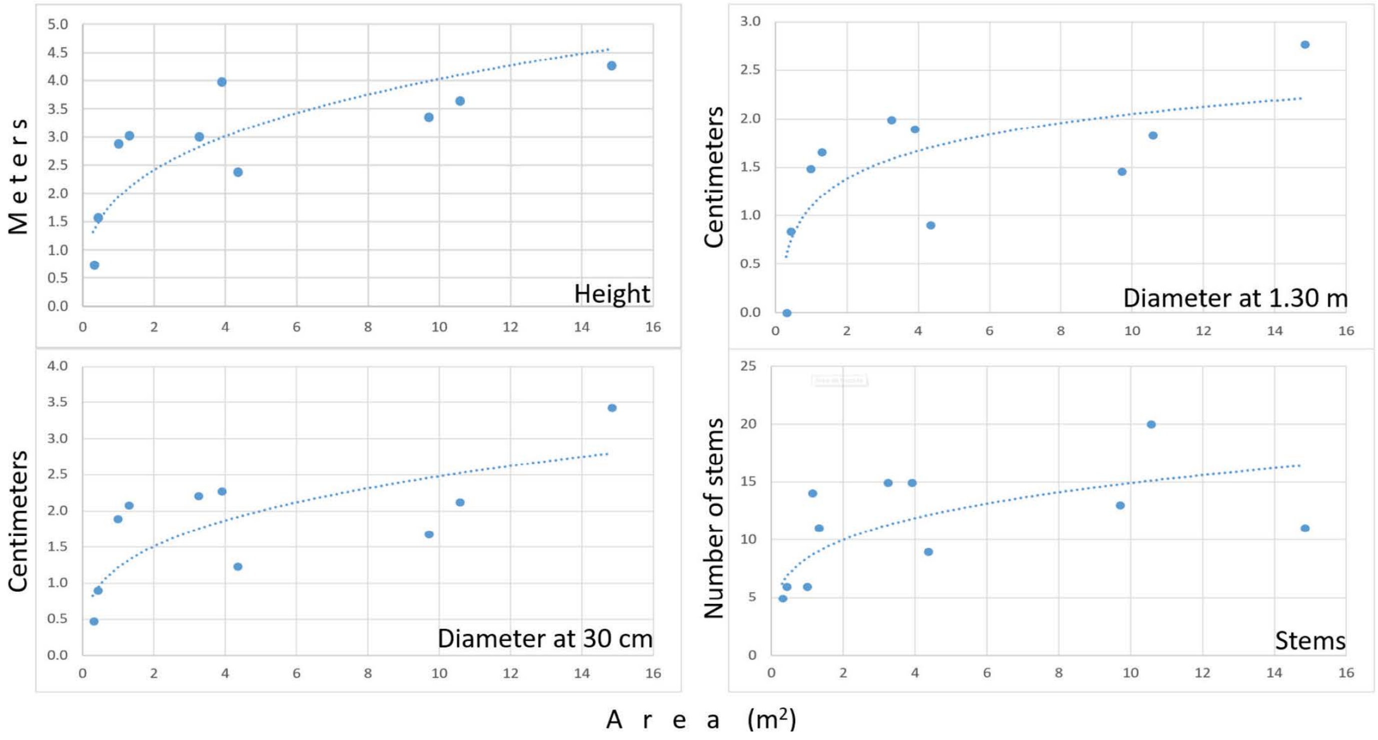

Figure 4. Trend of the relationship of areas of strains with several dasometric parameters.

The allometric equations corresponding to these trends are shown in Table 1, where the exponential models prevail, with the exception of the relationship of the area with diameter at 1.30 m, whose best adjustment was defined with a logarithmic model. On the other hand, the determination of the model's adjustment to estimate the dasometric parameters and the proportion of the variation of the results defined by the model, was rather low. However, the models were significant (p-value), with the exception of the model that estimates the number of stems, which implies that there is a significant relationship between the coverage area of the strain with the dasometric variables.

Estimation rate

As a result of the application of allometric equations, Table 2 shows a table for the estimation of the dasometric parameters. Based on this rate, it is enough to understand the area (m^2) of coverage of a bamboo strain, to be able to estimate, for example, the height or the diameter at 1.30 m. Therefore, the sum of the values of the individual strains allows making estimations of the productivity of the entire plantation, in a more practical way. However, it must be considered that because *Guadua* spp. presents an open sympodial rhizome growth, the use of allometric models generated in this study is restricted to this genus.

Table 1. Allometric equations for the estimation of parameters of *Guadua* spp. bamboo strains, in relation to their coverage area.

Variable	Equation	R^2	p-value
Height	$=1.94 * (\text{Area}^{0.317})$	0.67	0.0222
DAP	$=0.4146 * (\ln(\text{Area}))+1.0949$	0.56	0.0367
DC	$=1.2188 * (\text{Area}^{0.3085})$	0.57	0.0313
NC	$=8.4308 * (\text{Area}^{0.2471})$	0.54	0.1301

NC=Number of stems; DC=Diameter of stem at 30 cm; DAP=Diameter at 1.30 m.

Table 2. Estimation rate of dasometric parameters of bamboo strains, based on the coverage area.

Area (m^2)	Height (m)	DAP (cm)	DC (cm)	NC
1	1.940	1.095	1.219	8.431
2	2.417	1.382	1.509	10.006
3	2.748	1.550	1.711	11.060
4	3.011	1.670	1.869	11.875
5	3.231	1.762	2.002	12.548
6	3.424	1.838	2.118	13.127
7	3.595	1.902	2.221	13.636
8	3.750	1.957	2.315	14.094
9	3.893	2.006	2.401	14.510
10	4.025	2.050	2.480	14.893

DAP=Diameter at 1.30 m; DC=Diameter at 30 cm; NC=Number of culms.

CONCLUSIONS

The results showed that it was possible to determine the coverage area of individual strains of bamboo with the technology of unmanned aerial vehicles (UAVs). The estimation of dasometric parameters of bamboo strains is possible based on the allometric relationship with the area of crown coverage of these strains. The use of UAVs facilitates the capture of aerial images, of quality for the tridimensional digitalization of forest plantations. Through the use of a geographic information system, it is possible to associate forest parameters of the tree cover taken in the field with the georeferenced location of the existing and eliminated tree coverage. Processes were defined in a geographic information system to locate bamboo strains in a plantation, based on specific criteria, as for example: species, condition, area, height, number of stems, diameter, as well as combinations between these criteria. The use of UAV technology facilitates evaluation work and monitoring of the bamboo plantation, since it can help to keep a historical record of its condition, which can be consulted without the need to return to the site in the field.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of the concentration of the nutrient solution on the nutrient content of chrysanthemum (*Dendranthema grandiflorum* (Ramat.)

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ABSTRACT

Objective: Evaluate the nutrient concentration at the foliar level of chrysanthemums grown in tezontle sand using three concentrations of the Steiner Universal Nutrient Solution.

Design/Methodology/Approach: A completely randomized design was used, each treatment represented a concentration of the nutrient solution and each concentration had 11 repetitions, the study variables were: foliar concentration of nutrients: N, P, K, Ca, Mg and S, plant height (AP), stem diameter (DT), number of leaves (NH), flower diameter (DF), fresh weight (PF) and dry weight (PS).

Results: The results showed this order of extraction K>N>Ca>P>Mg>S, for all concentrations, the foliar N content was low in the three treatments (<3.35%), the P was found in high concentrations. (>0.63%), K was higher in the 100% concentration treatment, Mg (>0.44%) was in sufficient levels, in Ca the 100% concentration had greater absorption and finally the S content was only the adequate in the concentration of 100%, in the morphological variables there were no significant differences.

Limitations of the study/Implications: High temperatures helped the proliferation of pests; therefore, it would be good to have a better control of temperatures inside the greenhouse.

Findings/Conclusions: Therefore, using different concentrations of the nutrient solution in chrysanthemums grown in tezontle sand affects the nutrient content at the foliar level but not necessarily its morphology.

Keywords: leaf analysis, tezontle sand, Universal Steiner.

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INTRODUCTION

Mexico is considered one of the countries with the longest floricultural tradition due to its climate, soil, and labor characteristics, presenting growth potential in flower production (Flores-Ruvalcaba *et al.*, 2005). One of the most popular flowers is the chrysanthemum, native to East Asia; currently, the State of Mexico grows almost 90% of all the flowers sold in the country (Tejeda-Sartorius *et al.*, 2015). According to information from the Agro-Food and Fisheries Information Service (SIAP, 2020), chrysanthemums obtained 11 million 312 thousand 281 grosses, the State of Mexico contributed 1,587 million pesos, 87.8% of the

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national value, it should be noted that the State de Puebla has a planted area of 102.5 ha, which gives a production of 528,312.76 t, a yield of 5,154.27 t ha^{-1} with an average rural price of $174.32 per t.

On the other hand, we know that, for hundreds of years, agriculture in Mexico has offered a wide range of crops to satisfy human needs. However, in the case of food and industrial crops, the misuse of technologies, such as irrigation and fertilization, has caused such soil degradation that currently thousands of hectares cannot be cultivated. In the last sixty years, work has been done on the development of new systems that help solve these problems. Hydroponics is one of the alternatives according to Solís (2016) it is considered as an agricultural production system, which is of great importance within the ecological, economic and social context. Soilless crops are usually classified into hydroponic crops (grown in water plus nutrients or on inert materials) and substrate crops (grown on chemically active materials with cation exchange capacity). In addition, soilless crops can function as an open system, a lost solution (non-cyclic) or as a closed system, with circulation of nutrient solutions (San Martin, 2011). Among the substrate crops is the red, yellowish or black tezontle sand cultivation system of very porous volcanic origin, also known as volcanic tuff, abundant in various regions of Mexico, very popular as a horticultural substrate. It is considered a chemically inert material, it has a neutral to slightly alkaline pH, very low CEC, high total porosity, although its physical properties depend greatly on the size of the particles, it maintains its structure unchanged over time (Martínez & Roca, 2011).

Jiménez (2015) mentions that, in hydroponics, a large part of the success is to use the adequate nutrient solution, since the substrates and the water do not provide the necessary nutrients for the optimal development of the plants. A complete nutrient solution must have macroelements and microelements, Steiner Universal Nutrient Solution has a mutual relationship between the anions NO_3^-, $H_2PO_4^-$ and SO_4^-, and between the cations of K^+, Ca^{2+} and Mg^{2+}. It was since that a nutrient solution must be regulated in its macronutrients contained in the mentioned ions. The nutritive relationship consists not only in the absolute amount of each element provided but also in the quantitative relationship established between the anions on the one hand and the cations on the other. Steiner (1961) indicated that when the nutrient solution is applied continuously, plants can absorb ions at very low concentrations, however, it is likely that, at too low a concentration, the minimum demand for certain nutrients is not covered. Therefore, the objective of the present investigation was to evaluate the nutrient concentration at the foliar level of the chrysanthemum crop in tezontle sand using three concentrations of the Steiner Universal Nutrient Solution. The nutrient concentration at the foliar level has already been studied by several authors Osorio (2012), studied the adequate levels of soil fertility and foliar analyzes for chrysanthemum, establishing adequate levels for each element.

MATERIALS AND METHODS

This work was carried out in a 71.56 m^2 glass chapel-type greenhouse of the Graduate Program in Horticulture. It was during the period of December 2021 and ended in April 2022. It began with the sifting of the red tezontle substrate, to obtain granulometries of 2 to 4 mm (fine) and 5 to 8 mm (coarse), it was disinfected with sodium hypochlorite, leaving it to rest for 24 hours, after which time it was rinsed with water to avoid residues and allowed to dry. The pots that were used were recycled, with a diameter of 20 cm, height of 18 cm and a volume of 4.5 liters, perforated at the bottom to drain the nutrient solution, of the total volume only 3.5 liters of its capacity were used, placing them in 1/3 of the thickest substrate in the lower part and 2/3 of the fine substrate in the upper part, separated by tulle-type fabric, finally, they were placed on a base with a slope of 1% in three rows corresponding to the treatments. In addition, 3" PVC tubes cut in half were used to capture the drained nutrient solution, which was stored in 5-liter buckets for each treatment. The plant material used was a yellow pompom-type chrysanthemum. The nutrient solution was not recirculating. Based on the Steiner Universal Nutrient Solution, the respective calculations were made according to the three concentrations 85%, 100% and 115% with an EC of 2.2, 2.4 and 2.8 dS/m depending on the concentration, maintaining a pH of 6.0-6.5. The irrigations were automated with the use of analog timers, each hour there was an irrigation with a duration of 15 minutes distributed by means of tapes with drippers every 20 cm that threw 286 ml per minute, therefore a nutrient solution was prepared every third day once the experiment has been set up. During the first six weeks, night lighting was provided from 7:00 p.m. to 01:00 a.m. the following day with 100-watt incandescent light bulbs. After the second week, the weeding activity was carried out, with crop management. The experimental design used was completely randomized (DECA), three concentrations of Steiner Universal Nutrient Solution (85%, 100% and 115%) were evaluated, each with 11 repetitions. The variables that were evaluated were the foliar concentration of the elements: nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, taking the foliar samples before harvest. The morphological variables were taken during the harvest: plant height (AP), stem diameter (DT), number of leaves (NH), fresh weight (PF) and dry weight (PS), root, stem + leaves and flower. Subsequently a means test was performed with the Tukey test ($P \leq 0.05$).

RESULTS AND DISCUSSION

After analyzing the data, the following results were obtained:

Table 1 shows the results of the means tests of the morphological variables AP: plant height, NH: number of leaves, DT: stem diameter and DF: flower diameter, noting that there are no significant differences ($P \leq 0.05$) between concentrations. Numerically, the plants supplied with the 115% nutrient solution reached higher AP, in the NH the concentrations of 100 and 115% had one more leaf than those of 85%, the DT was higher in the 85% concentration reaching 0.79 cm, for Finally, the DF was higher in the concentration of 100%.

In the following Table 2, the values of (PF) fresh weight and (PS) dry weight are observed, which also do not present significant differences, numerically there was a higher total PF in the most concentrated nutrient solution (115%) and greater accumulation of dry matter in the lowest concentration (85%).

Analyzing the data obtained from the laboratory samples Table 3, the nitrogen content was higher in the concentration of 115 %, however, the levels fall below the intervals proposed by Osorio (2012), despite that they were not observed. deficiency symptoms. It is

Table 1. Comparison of means of the three concentrations of the Steiner Solution on the morphological variables: AP, NH, DT, DF.

Concentration of the nutritive solution (%)	AP cm	NH cm	DT cm	DF cm
85	72.72 a	23 a	0.79 a	6.83 A
100	71.95 a	24 a	0.77 a	6.96 A
115	82.58 a	24 a	0.76 a	6.86 A

AP: plant height, NH: number of leaves, DT: stem diameter, DF: floral diameter. Values with the same letter within the columns are equal according to Tukey's test at P≤0.05.

Table 2. Comparison of means of different concentrations of the Steiner Solution on the fresh weight and dry weight of different organs of the chrysanthemum plants.

Organ of the plant		Stem+leaves		Root		Flower	
		PF g	PS g	PF g	PS g	PF g	PS g
Concentration of the nutritive solution	85%	56.875[a]	16.245[a]	18.168[a]	7.725[a]	13.853[a]	1.86[a]
	100%	52.128[a]	14.62[a]	23.203[a]	7.058[a]	15.764[a]	1.9275[a]
	115%	55.94[a]	14.758[a]	21.098[a]	7.378[a]	14.968[a]	2.08[a]
DMS		18.933	5.7347	10.5	5.155	3.6347	0.4764

Values with the same letter within the columns are equal according to Tukey's test at P≤0.05. PF: fresh weight, PS: dry weight, DMS: minimum significant difference.

Table 3. Nutrient content in yellow pompon chrysanthemum leaves due to the concentration of the nutritive solution.

Concentration of the nutritive solution (%).	Nitrogen %	Phosphorus %	Potasium %	Sulfur %	Calcium %	Magnesium %
85	2.18 B	0.69 A	7.4 S	0.24 B	2.28 S	0.38 A
100	3.5 B	0.63 A	8.55 S	0.37 A	2.38 S	0.46 A
115	3.08 B	0.86 A	5 A	0.16 B	1.98 A	0.32 A

Data with letter B represent low levels, with letter A high levels and with letter S they represent sufficient values, based on what was obtained by Osorio (2012): N (4.5-5.5), P (0.3-0.6), K (4.0- 6.5), S (0.3-0.7), Ca (1.0-2.0), and Mg (0.3-0.6Data in red color represent low levels, blue high levels and black sufficient, based on what was obtained by Osorio (2012): N (4.5-5.5), P (0.3-0.6), K (4.0-6.5), S (0.3 -0.7), Ca (1.0-2.0), and Mg (0.3-0.6).

said that the deficiency favors the lignification of the stems which reduces the flow of water after the harvest, thus decreasing the vase life.

The phosphorus content was higher in the treatment with the concentration of 115%, the three treatments having a sufficient to very high concentration, a restricted availability of phosphorus produces shorter and/or more compact plants.

The most absorbed nutrient by the plant was K, reaching up to 8.55% in the 100% concentration, Anuradha *et al.* (1990), mentions that the increase in potassium extraction before harvest may be due to its participation in the synthesis of anthocyanins and other pigments, producing a greater intensity of the color of the inflorescence in cempasúchil

(*Tagetes erecta* L.). Sulfur was higher in the 100% concentration, but in the 115% concentration the levels fell below those required. In the plant, sulfur is concentrated in greater quantities in mature leaves than in young leaves, this is due to its greater metabolic maturity, which probably could have happened, since the leaves from the middle part of the plant were taken (INTAGRI, 2001).

The calcium content at the foliar level was found to be high to sufficient in the three treatments, being higher in the concentration of 100%, followed by that of 85% and finally that of 115%. Regarding magnesium, optimal levels were obtained, its concentration at the foliar level is sufficient for the three treatments. The concentration of 100% and 85% presented a higher percentage, although these data agree with those obtained by Pineda *et al.* (1998) and Carrillo (2009), who reported higher levels in the treatments with lower concentrations in chrysanthemums.

CONCLUSIONS

In the morphological variables, as well as in the fresh weight and dry weight, there were no significant differences ($P \leq 0.05$) between the concentrations of the nutrient solution. Instead, the foliar analysis reflected better absorption of most of the nutrients in the 100% concentration, except for phosphorus. An effect of the different concentrations of the nutrient solution on the foliar nutrient content was found, but this did not alter the morphological characteristics of commercial interest in the chrysanthemum plants.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Peri-urban home gardens in San Cristóbal de Las Casas, Mexico are fundamental spaces of resistance

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ABSTRACT

Objective: To determine the effects of neoliberal policies (including the Green Revolution), the urban sprawl (as a consequence of population growth), and the reduction of agricultural areas on peri-urban agriculture; however, the main focus is the forms of resistance against these pressures from the dominant system.

Design/Methodology/Approach: Through participant observation, surveys, collection, and botanization we identified plant species, their diversity, uses, and richness. Home gardens in San Felipe Ecatepec, Chiapas, Mexico are a system, which consists of subsystems, functions, composition, and management, as well as a high number of species, high to moderate richness, and a surface that oscillates between 600 m^2 and 2500 m^2. Growing products next to the house provides healthy and fresh food, creates a useful and productive space, and preserves agrobiodiversity. It is an agroecosystem where each family and sitio or home garden interact with other families and other home gardens, integrating local knowledge and offering a space for families to live together. They can be considered spaces of resistance based on traditional knowledge, which also help to control their resources and to bolster individual and collective food sovereignty.

Results: Home gardens in San Felipe Ecatepec, Chiapas, Mexico are a system, which consists of subsystems, functions, composition, and management, as well as a high number of species, high to moderate richness, and established on surfaces that oscillates between 600 m^2 and 2,500 m^2.

Study Limitations/Implications: The research was carried out during the two years of the COVID-19 pandemic, which posed an extra challenge to the field work.

Findings/Conclusions: Growing products next to the house provides healthy and fresh food, creates a useful and productive space, and preserves agrobiodiversity. It is an agroecosystem where each family and sitio or home garden interact with other families and other home gardens, integrating local knowledge and offering a space for families to live together. They can be considered spaces of resistance based on traditional knowledge, which also help families to control their resources and to bolster individual and collective food sovereignty.

Keywords: Urban agriculture, food sovereignty, agrobiodiversity, agroecology.

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INTRODUCTION

Current food systems (*i.e.*, the agro-industrial model) are not sustainable. The said model (also known as the Green Revolution) has prevailed in the Latin American region for decades, promoted and defended by neoliberal governments. The agri-food companies are the sole beneficiaries of the agro-industrial model and its crisis. The advance of this

Image by Alicja at Pixabay

model has been supported by many local, regional, state, or international governments and administrations or public institutions, which prioritize the commodification of agriculture and food. Policies are market driven and profits are based on speculation and the maximization of business profits (Escobar Moreno, 2006; FAO, 2014; Gómez Martínez, 2015; Barzola, 2019; GANESAN, 2020; MSCPI, 2021).

This model is also responsible for around 1/3 of greenhouse gases and 80% of biodiversity and agrobiodiversity losses. According to the Food and Agriculture Organization of the United Nations (FAO), approximately 75% of plant varieties and animal breeds have been lost since 1900, narrowing the genetic diversity on which human diet is based (Goome, 2008). In addition to the use of toxic inputs in production, this model encouraged the loss of traditional knowledge and promoted the ultra-processing of food. These policies have sustained social inequality and the loss of cultural values (Ceccon, 2008; Gómez Martinez, 2015, Barquera *et al.* 2020; Benítez *et al.*, 2020).

Therefore, the right of peoples to define and have control of their food and food production systems must be restored and strengthened, in both the local and the national spheres (ALAI, 2016). Home gardens provide such an opportunity, as a starting point whose keepers continue to produce and maintain ancestral practices and folk wisdom. They are the reflection of this traditional agriculture model, which has resisted all the policies that have tried to end it, considering it obsolete. This model is based on family workforce and organization, as well as on the use of resources, traditional knowledge, and manual instruments, surviving the attack of the agro-industrial model. This type of agriculture has been marginalized, has received little support, and has been discredited. Nevertheless, it still sustains food worldwide (Suárez Carrera, 2016).

This agriculture is mainly supported by the population that owns less than 5 hectares and depends on agriculture, day labor, and other salaried work. Their land is mainly use for rainfed agriculture and its production is destined for self-sufficiency and the surpluses for the domestic or export market.

Traditional agriculture originally maintained food sovereignty; this phenomenon is still ongoing in some regions —for example, in the home gardens studied. This culture still resists in these spaces. Other interesting stories can be found in the research carried out by the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO 2022) about how peasants in 7 different regions of Chiapas have preserved their milpas. They provide another example of how this ancestral production system provides a space of resistance that preserves lore and traditions and protects native seeds which future generations will be able to sow.

Food should be once more produced according to the specific socio-environmental conditions of each place, respecting the natural systems and the social, cultural, and technological activities that peasants have practiced throughout history (Nuñes, 2000).

The impact of the aforementioned models has not left the municipality of San Cristóbal de Las Casas, in Chiapas, Mexico, unscathed: the urban sprawl and real estate development have spread through the valley of the municipality —where the community of San Felipe Ecatepec is located. These phenomena have increased the demand for public services; likewise, they have led to great environmental and social challenges.

In many places, the home gardens, plots, or *sitios* (as they are called in San Felipe Ecatepec) remain the primary source of food supplies that satisfy family needs and favor food sovereignty, as the handiest resource to survive the environmental risk and the market contingencies or fluctuations (Mariaca, 2012). The home gardens of San Felipe Ecatepec are no exception: they still put in practice local knowledge and preserve agrobiodiversity through diversified production and self-production of food. These sitios resist public policies that have favored both the development of agriculture with highly toxic inputs and urban development policies, which ultimately allocate lands to housing development, to the detriment of their agricultural potential.

Therefore, research was carried out to identify peri-urban home gardens and to determine their characteristics and how they carry out their activities. The home garden was studied as a system with subsystems that have certain functions, composition, and management. Our objective was to determine the diversity and use of the species that they manage. The field work was likewise focused on the social function that the home garden has in the community and fresh food consumption (Barbosa y Fonseca, 2019).

From the social aspect, considering the vision of Craviotti and Pardías (2012), the growth of urban areas and the pressure they put on rural systems, as well as the capacity for action of the subjects, are generating collective forms of resistance, rooted in shared traditional lore and dense social networks. This phenomenon is particularly based on the communal nature of the land, which cannot be sold to people outside the community. This resistance can be seen in the ongoing tradition of growing crops next to the house. Peasants continue with this activity, despite the pressure of urban sprawl and population growth, which influences both agriculture and home gardens, as well as increasingly smaller food production areas. Consequently, agricultural products decrease. This has also forced families to join urban activities in order to meet their needs; however, the ancestral culture of maintaining the home garden still provides them with fresh and healthy food.

The results are a relevant contribution to the study of peri-urban home gardens, which may serve as a basis for further research on the subject. It can also serve as an instrument for decision-making at different government levels, such as the whole state of Chiapas or the Desarrollo Rural and Desarrollo Urbano offices of the municipality; it can also support the decision-making of the social sector, including peasants or organizations that are working with home gardens and urban street markets.

MATERIALS AND METHODS

The study area is in the physiographic region of Altos de Chiapas, in the Altos Tsotsil Tseltal fifth socioeconomic region. It is located about five kilometers to the west of San Cristóbal de las Casas, Chiapas, Mexico (Camacho *et al.*, 2007), at a latitude of 16° 43' 31.61" N, a longitude of 92° 40' 28.99" W, and an average elevation of 2,223 m.a.s.l. The relief ranges from 1,000 m to 2,800 m above sea level and consists of a high mountain chain with stretched slopes (76.77%), a stepped plateau with low hills (19.61%), an intermontane valley (3.62%). The climates that have been recorded in the municipality are: temperate subhumid with dry winter (82.05%), semi-warm subhumid with dry winter (12.47%), temperate humid with no dry season (5.47%), (INEGI, 2020). The vegetation cover in the municipality consists of pine-oak forest and secondary vegetation. The types of soils recorded in the municipality are: Luvisol (67.42%), Alisol (17.23%), and Gleysol (2.05%).

The original name of San Felipe Ecatepec was "Muk Ti Nam", which means "on the shore of the large lagoon". When the Spanish conquistadors arrived in the 16th century, the place was inhabited by people from the Maya Tsotsil group.

The participant observation technique (Amezcua, 2016) was used for this research. In the first stage, the study area was the subject to a reconnaissance visit and we established contact with the community authorities. The objectives of the research were explained to an Assembly and authorization was requested from the community commissioner to carry out the field work. Subsequently, visits were made to determine the existence and location of home gardens, and to identify the families that were interested in collaborating in the study. As a result, 10 home gardens were chosen.

In the second stage, we worked with representative people; we listened to them and verified their input by asking another member of the family or community producer, in order to obtain and complement different visions. The reports obtained were compared with our observations, recording our observations, perceptions, and scenarios in the field notebook. Questionnaires with closed and open questions were applied to the owners of the home gardens.

To calculate the home gardens' species richness, the individuals were counted, using the Margalef index (MI) proposed by Funes (2009), which includes crop, tree, and domestic animal species. The common names and uses of all the species of plants present in the home gardens were recorded with the participation of the home gardens' owners. The uses heading included the following categories: edible, medicinal, fodder, construction, ceremonial, fuel, fence, ornamental, and other. Plants were photographed, collected, and botanized for future identification. The plants that could not be recognized in the field, were photographed, and specialized bibliographic material, information available on the Internet —such as the International Plant Names Index (IPNI, 2022), Tropicos (ORG 2021), and Enciclovida (CONABIO, 2016)— were used for comparative and identification purposes.

Finally, the information obtained was analyzed, the qualitative information was systematized, and the texts of the interviews were examined.

RESULTS AND DISCUSSION

The family subsystem in San Felipe Ecatepec is made up of the nuclear and single parent types. Nuclear families are the traditional type of family; they include two generations (parents plus children); they are extensive or complex (three or more generations: parents, children, grandparents, and great-grandparents). Meanwhile, single parent families are made up of a mother plus children (Roman *et al.*, 2009). Seven women and three men are in charge of coordinating activities in these spaces. Their ages range from 44 to 70 years. Most of them attended primary and secondary school and the children of some producers have already studied an undergraduate degree. They speak only Spanish, no one has migrated to another country, and they are Catholic.

The families own the homes in which they live. Their houses are built with concrete blocks, cement slab, and stone; two of them are built with mudbrick and reed. They are connected to the power grid. Nine have access to well water and one person buys water from a pipe. None of the ten home gardens receives drinking water supply from the municipal system (SAPAM). Eight households cook with different fuel sources (butane gas, electricity, firewood, coal) and two use butane gas and electricity. Eight houses have access to the sewerage system, one uses a septic tank, and only one family uses a latrine to deposit excrement.

The ten families separate the organic and inorganic waste. The organic waste is sent to the *sitio* or home gardens and the inorganic waste is picked by the collection truck that circulates through the main streets. Secondary streets are dirt roads, while the main streets are paved.

Only two people mentioned that they receive support from government programs. Seven obtain their economic income from salaried work and three from local commerce.

The heads of the family (both women and men) carry out the following activities: domestic worker, mechanic, employee in the hotel sector (as temporary employees in the laundry area), construction work, carpentry work, sale of prepared products, confectionery, dressmaking, and elaboration of decorated paraffin candles. These jobs are carried out either in the community or in the municipality.

The domestic units have different spaces, with different land-uses and production systems (*e.g.*, milpas, vegetable gardens, *acahuales*, or forests), most of which are located in the back of the house or, in some cases, around it.

All these elements make up the home garden, which families call sitio and describe it as the place where they have their fruit trees, animals, milpas, ornamental and medicinal plants, among others. The house is integrated and interacts with the home garden or sitio, as well as with other gardens, milpas, ecosystems (such as the temperate pine-oak forest), and institutions, such as the municipal agency, the office of the commissioner, and the market (Figure 1).

These agroecosystems includes short-cycle plants —corn (*Zea mays*), broad beans (*Vicia faba*), and beans (*Phaseolus vulgares*)—, evergreen and deciduous fruit trees —Mexican hawthorn root (*Crataegus mexicana*), common quince (*Cydonia oblonga*), black cherry (*Prunus serotina*), or avocado (*Persea* spp)—, multipurpose plants —Mexican elder (*Sambucus mexicana*), Mexican pepperleaf (*Piper auritum*), and spineless yucca (*Yucca elephantipes*)—, medicinal plants —citrus scented marigold (*Tagetes nelsonii*), sweet fennel (*Foeniculum vulgare*), and dandelion (*Taraxacum official*), and ornamental flowers —calla lily (*Zantedeschia aethiopica*), bigleaf hydrangea (*Hydrangea macrophylla*), and belladonna lilies (*Amaryllis* spp). Only a few of the species recorded in the home gardens are mentioned here.

The land is communal. People practice rainfed agriculture and cultivate using hand tools. The land destined for cultivation is flat and only two home gardens have a slight slope. The smallest property has an area of 600 m^2 and the largest measures 2,500 m^2. Regarding their richness, eight of the ten home gardens or sitios have a high biodiversity, while in the other two it is moderate (Figure 2).

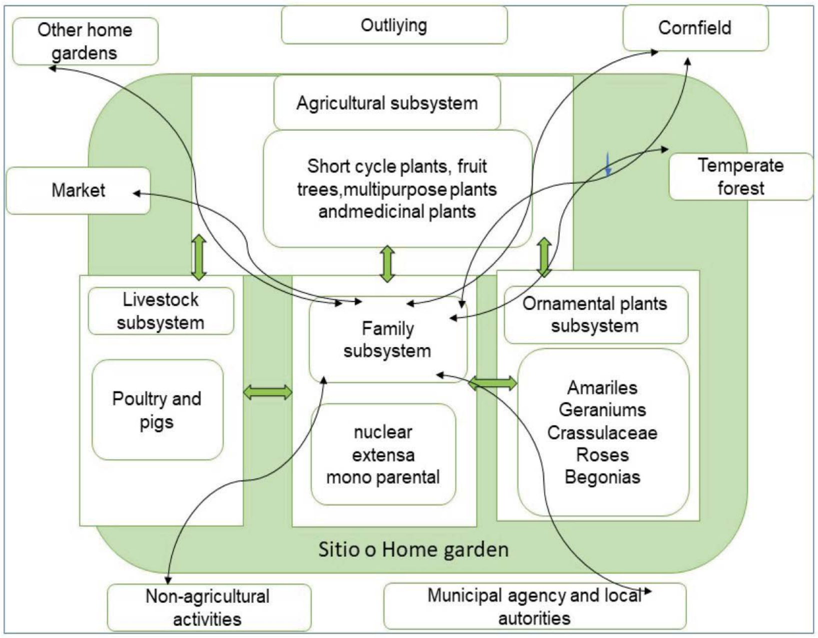

Figure 1. Home gardens, subsystems, and their relationships. Developed by the authors (2022).

Family garden	Species	Number of individuals
GF1	87	926
GF2	21	737
GF3	69	416
GF4	76	421
GF5	39	2295
GF6	65	1591
GF7	53	619
GF8	56	242
GF9	152	2149
GF10	64	250

Figure 2. Wealth of small backyard plants and animals from the ten home gardens or sites.

Plants provide food, medicine, fuel and oxygen; they regulate humidity and contribute to the stability of the climate. Figures 3 and 4 show the number of species and the uses in the ten home gardens.

A remarkable aspect of this research is that was carried out during the two years that the COVID-19 pandemic lasted. The interviewees identified several of the plants in the home garden as ingredients for home remedies that helped them cope with the effects of the disease. Additionally, the families and the research team maintained sanitary measures at all times to avoid contagion; likewise, the research team respected the times that they were asked not to visit their homes, because family members had been infected. It is worth emphasizing that nobody among the families that were part of the study died

Garden	1. Edible plants	2. Medicinal plants	3. Forage	4. Building	5. Ceremoniales	6. Firewood	7. Siege	8. Ornamental	9. Others
1	39	23	3	0	0	0	1	46	8
2	12	2	1	2	0	7	2	2	0
3	27	12	0	1	0	6	6	33	9
4	32	27	2	2	0	5	6	37	9
5	25	10	1	1	0	3	5	12	2
6	28	14	3	4	1	5	7	24	2
7	24	5	1	4	0	5	10	23	2
8	26	9	1	1	0	4	2	28	1
9	48	9	1	1	0	3	2	102	2
10	22	16	2	0	0	4	2	34	0
Totales	283	127	15	16	1	42	43	341	35

Figure 3. Knowledge and use of plants in ten Family Gardens.

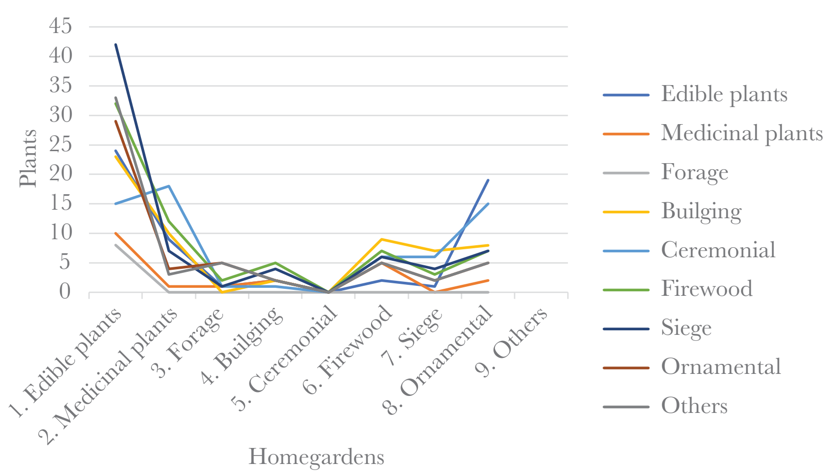

Figure 4. Graph of knowledge and uses about plants.

as a consequence of the disease. Although these families are linked with urban areas for several reasons (jobs, sale of products, among others), we could infer that their lifestyle and relationship with plants, as well as the possibility of being outdoors, lessened the impact of the pandemic with regard to urban areas.

Population growth and the expansion of the urban sprawl have an impact on agriculture and home gardens, as a result of the reduction of the areas destined for food production. Families are forced to participated in urban dynamics, in order to diversify their monetary income. Changes in land-use and housing land market have a major impact on agricultural land (Calderón-Cisneros and Soto-Pinto 2014).

The home gardens of San Felipe Ecatepec have an ongoing interaction with other agroecosystems. We infer that, to a certain point, the communal nature of the land influences their persistence.

The home garden (hf9) manages 152 species and has an area of 600 m^2, while the largest home garden measures 2,500 m^2 and has 39 species. In other words, the number of species does not depend on the land area. Eight sitios have a high biodiversity and two have a moderate diversity; this phenomenon is directly related to the jobs that the families do, the time they have available, and how they are organized.

The home gardens or sitios are considered as spaces of social resistance, because they hold back the growth of urban areas and the pressure they exercise on rural systems. The capacity for action of the subjects is generating collective resistance forms, rooted in a shared traditional lore and in dense social networks (Craviotti and Pardías, 2012). Meanwhile, Hernández and Sánchez (2010) also identified urban vegetable gardens in different parts of Spain as a collective process of resistance and articulation of alternative food proposals. Nevertheless, it refers to the resistance and recovery of the urban agriculture space (landscape, territory, mode of production, way of life, etc.). This phenomenon is linked to a counter-hegemonic neighborhood movement, based on questioning the control of the subsistence means by the globalized agri-food system and the recovery of the satisfiers, in order to satisfy human needs. Obtaining and producing food become an act of daily resistance. Therefore, as part of the elements of resistance aimed at sustainability, agroecology is the theoretical approach that sets down the guidelines for the ecological management of natural resources, the environmental issue, political ecology, and the technical, economic, social, and cultural aspects of social change towards a more sustainable society (Hernández and Sánchez, 2010; Sarandon and Flores 2014).

Likewise, although on a larger scale and in the context of the commodification of agriculture and the resistance against soybean agrarian extractivism, Barzola (2019) identifies how the resistance of the "Malvinas Lucha por la Vida" collective in Argentina confronted the multinational Monsanto and managed to expel it from its territory. This experience of struggle and resistance highlighted the crisis of the civilizing and productive model; consequently, the community did not only revalue their territory, but they also discussed the need for an alternative agricultural model that respects the food sovereignty of the peoples.

Consequently, on a small or medium scale, the resistance to the agro-industrial model or the neoliberal policies that have been implemented for more than half a century can lead to the maintenance or rescue of healthy food production systems that respect the cycles of nature and ecosystem relationships.

CONCLUSIONS

The home gardens of San Felipe Ecatepec are the primary source of healthy food. They are not isolated; on the contrary, they interact with other subsystems. These peri-urban agroecosystems are productive and highly diverse, they contribute to the conservation of agrobiodiversity, and they influence the food sovereignty of families living in peri-urban areas. The land is communal, which in a certain way has favored the permanence of these production systems.

In this process of urban sprawl and its pressure on rural systems, the subjects' capacity for action is generating collective resistance, rooted in shared traditional kowledge and supported by dense social networks. This phenomenon is enhanced because the land is communal and cannot be sold to outsiders. This resistance can be perceived in the way that this tradition (cultivating next to the house) is maintained, as a mechanism that helps them confront the hegemonic development model.

They continue to do this despite the pressure of urban sprawl and population growth, which influences both agriculture and home gardens, as well as increasingly smaller food production areas, which causes a decrease in agricultural products. This has also led families to participate in urban activities to meet their needs. However, the ancestral culture of having a home garden that provides them with fresh and healthy food persists.

Several agroecological practices that are implemented to different crops contribute to the production and consumption of healthy food, likewise helping to reverse the causes of low yield and reducing high input costs.

This productive system can be considered as a space of resistance. It is supported by traditional culture or lore, allowing families to increase their control over their resources, both individual and collective. The home garden space also serves as a family meeting place, where they share hours of conversation, joint work, food consumption, and leisure. However, one traditional factor puts this agroecological system at risk: the distribution of land in inheritance. This threat has been reported since the beginning of the 20th century by the Food and Agriculture Organization of the United Nations (FAO) (2002). However, this should provide the basis for further studies that can follow-up these processes.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Main technical-productive characteristics of meliponiculture in two locations of the municipality of Calkiní, Campeche

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ABSTRACT

Objective: To identify the main technical-productive characteristics of two meliponiculture production systems —traditional and technical— in two locations of the municipality of Calkiní, Campeche.

Design/Methodology/Approach: Semi-structured interviews of 60 items were applied to bee growers from Pucnanchen and Santa Cruz between August and December 2021. The population of meliponiculturists was established by using the snowball method.

Results: We found a total of 53 bee colonies of the *M. beecheii* species (39 hobones and 14 technified boxes). The interviewed meliponiculturists are over 49 years old and have an average of 13.25 colonies per meliponary. In modern meliponaries, galvanized metal sheets have replaced huano (*Sabal yapa*) leaf roofing. Likewise, some *hobones* have been replaced by technified boxes to breed native bees.

Study limitations/Implications: This study describes the technical-productive characteristics of the two production systems (traditional and technical) used in meliponiculture in two locations of the municipality of Calkiní, Campeche.

Findings/Conclusions: There is a decline in the number of people practicing meliponiculture, an activity still rooted in rural communities and that retains its economic, cultural, and social importance. In rural communities, the use of melipona honey for health purposes persists, as well as the religious customs associated with this type of honey.

Keywords: Meliponiculture, Production methods, *Melipona*, *Scaptotrigona*, Calkiní.

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INTRODUCTION

The term meliponiculture refers to the activity of caring for and breeding native stingless bees (Tribe: *Meliponini*; Nates-Parra and Rosso, 2013; Quezada-Euán *et al.*, 2001; Quezada-Euán, 2005). In Mexico, meliponiculture is a cultural, economic, and social activity that was developed by Maya communities, with their own technology, before the arrival of European colonizers (Quezada-Euán *et al.*, 2001). Native stingless bees were cultivated in four areas, namely: 1) the Yucatán Peninsula; 2) the Gulf of Mexico coast (mainly Veracruz and Tabasco); 3) the Pacific coast (between Sinaloa and Jalisco); and 4) the Balsas River Basin in Guerrero and Michoacán (González-Acereto, 2012; Reyes-González, 2015).

Around 16 species of native stingless bees are present in the Yucatán Peninsula. However, the primary species used for honey production in Maya communities are *Melipona beecheii* and *Scaptotrigona pectoralis* (Quezada-Euán, 2011). The Maya peoples marketed the honey and cerumen produced by melipona bees to various regions, from the current border between Campeche and Tabasco to Guatemala and Honduras (Pereira-Nieto, 2005). The commercialization of honey was part of the sociocultural, medicinal, ritualistic, and food-related customs (it was used as a sweetener) of many indigenous peoples (Quezada-Euán, 2005; Carrillo-Magaña, 2004). Between the third and tenth centuries, when the Maya culture was at its peak, honey and cerumen were two of the most abundant trade products, along with salt, dried fish, *henequen*, cloth, and *copal* (Quezada-Euán, 2005; Carrillo-Magaña, 2004). The Maya refined their production methods to a level similar to that of the honey bee management in medieval Europe, reaching densities of up to 500 *hobones* (Quezada-Euán, 2011).

In the Maya culture area, especially in the Yucatán Peninsula, meliponiculture reached heights not found in other parts of Mexico. This was largely due to the development of various management processes such as meliponaries and hobones (Pat-Fernández *et al.*, 2018; Quezada-Euán, 2011; Carrillo-Magaña, 2004). The meliponary or bee house, known in the Mayan language as *Najil kaab*, is a simple structure supported by piles of solid wood, either jabín (*Piscidia piscipula*) or chacté viga (*Caesalpinia platyloba*), and covered with a roof made of Xa'an huano (*Sabal yapa*). The *Najil kaab* is built on the grounds behind the main house, usually with an east-west orientation. This location allows the families to have easy and quick access to the meliponary in order to supervise the bee colonies (Pat-Fernández *et al.*, 2018). Native stingless bees build their nests in pre-existing cavities (hollow trees, termite mounds, underground), using cerumen (wax mixed with resins), mud, and other materials. Brood combs are arranged horizontally or in clusters; honey and pollen are stored in ellipsoidal pots (Nates-Parra and Rosso, 2013). The indigenous peoples housed the bees in the well-known *hobones* or hollow logs where the melipona bees lodge. The *hobones* were placed horizontally so that meliponiculturists could extract honey from the sides (Quezada-Euán, 2011; Carrillo-Magaña, 2004).

According to Contreras-Escareño (1999), there are three different types of management systems for native stingless bees:

a) Rustic farming. Some inhabitants of the Yucatán Peninsula know the needs of melipona bees by direct observation. They open the hives once a year to harvest honey and do not conduct any other type of management. In rustic farming, there is no consistency in management or knowledge, even within the same community.
b) Traditional farming. This system is peculiar to towns with an ethnic history, where bee colonies are housed in containers—either pots or boxes (cardboard or wood) sheltered near their homes. In this case, there is consistency in the use of containers. For example, the Totonaca peoples use wooden boxes, the Maya use hollowed-out trunks, and the Nahua, clay pots.

c) Technified farming. This beekeeping system is conducted in artificial or rational housing. It uses wooden boxes to house native stingless bees. Said boxes are built respecting the architectural characteristics of natural nests and allowing for rational exploitation.

Currently, there is little information about the typology of producers. A lack of homogeneity has led to the formation of clearly differentiated groups. However, this situation facilitates the proposal of more precise public policies, whose effects in the short and medium term may help to achieve goals oriented towards technical and social improvements.

Farming of native stingless bees in Mexico has multiple economic benefits. Nevertheless, the peasants who obtain the products directly from the colonies face income-related obstacles due to their limited marketing channels. Moreover, they deal with problems related to the small amount of product collected per colony, which prevents them from increasing the market demand (Quezada-Euán, 2009). The aim of our study was to identify the main technical-productive characteristics of two meliponiculture production systems —traditional and technical— in two locations of the municipality of Calkiní, Campeche.

MATERIALS AND METHODS

Study area location

The study was conducted between August and December 2021. A semi-structured interview was created based on a schedule and applied to meliponiculturists of two localities: Pucnanchen and Santa Cruz. Both of them belong to the municipality of Calkiní, Campeche, Mexico (89° 53" and 90° 29" W, 20° 10" and 20° 51" N, at an altitude of 10 masl). Both locations were selected based on reports indicating that meliponiculture is practiced there (Pat Hernández *et al.*, 2018). We also used an observation guide to identify the environment of the meliponaries.

Instrument

The interview schedule comprised 60 items —closed and open-ended questions— organized into the following sections: family information, technical-productive characteristics, economy, and organization. To identify melipona honey producers we used the snowball method principles.

Variables

The applied instrument comprised the following sections: a) information on the family nucleus (family members, age, years of schooling); b) technical-productive characteristics (number of colonies per meliponary); c) origin and type of accommodation of the melipona bee colonies; d) characteristics of the meliponaries (roof, floor, and measurements); e) length of the hobones; f) techniques used in honey harvest and production volume; g) economic characteristics (retail price, destination, and form of commercialization of the harvested honey); h) and, finally, organization (form of association, number of partners, and seniority).

Recording and analysis of data

The information obtained through the interview schedules was recorded in an Excel spreadsheet (Microsoft Office®), and then analyzed using parameters and indicators of descriptive statistics (bar graph), as well as measures of central tendency and dispersion (mean and standard deviation).

RESULTS AND DISCUSSION

Maya meliponiculture is a pre-Columbian practice that subsists in some communities in the State of Campeche. Unfortunately, it is at serious risk of disappearing (Negrín-Muñoz and Sotelo-Santos, 2016). In this study, two meliponaries were selected in the town of Santa Cruz and another two in Pucnanchen to contrast production in both localities. Three of the four meliponaries are in private property, each one owned by a single person, and the remaining meliponary belongs to a working group of five women. The three meliponiculturists speak the Mayan language and are *ejidatarios* (members of an *ejido*, a communally owned piece of land). The women in the working group understand the Mayan language but do not speak it. The meliponiculturists are over 49 years old: 50, 60, and 92, respectively. Meanwhile, the working group has been functioning for three years, with meliponiculture as their main economic activity, followed by agriculture and the sale of their labor. According to Pat-Fernández *et al.* (2018), 30 peasant families in the Los Petenes Reserve are dedicated to the breeding of melipona bees, 13 of which live in Tankuché, seven in Pucnachén, four in Chunkanán, three in Concepción, two in Ex-Hacienda Santa Cruz, and one in Santa María.

Number of colonies per meliponary, origin, and accommodation type

We counted a total of 53 bee colonies of the species *M. beecheii* (39 hobones and 14 technified boxes), 25 of which were inherited, 14 were purchased, and 12 were extracted directly from the fields (Figure 1a; 1b).

The colonies are distributed in four meliponaries, with an average of 13.25 colonies per meliponary. According to Pat-Fernandez *et al.* (2018), meliponiculture in the Petenes

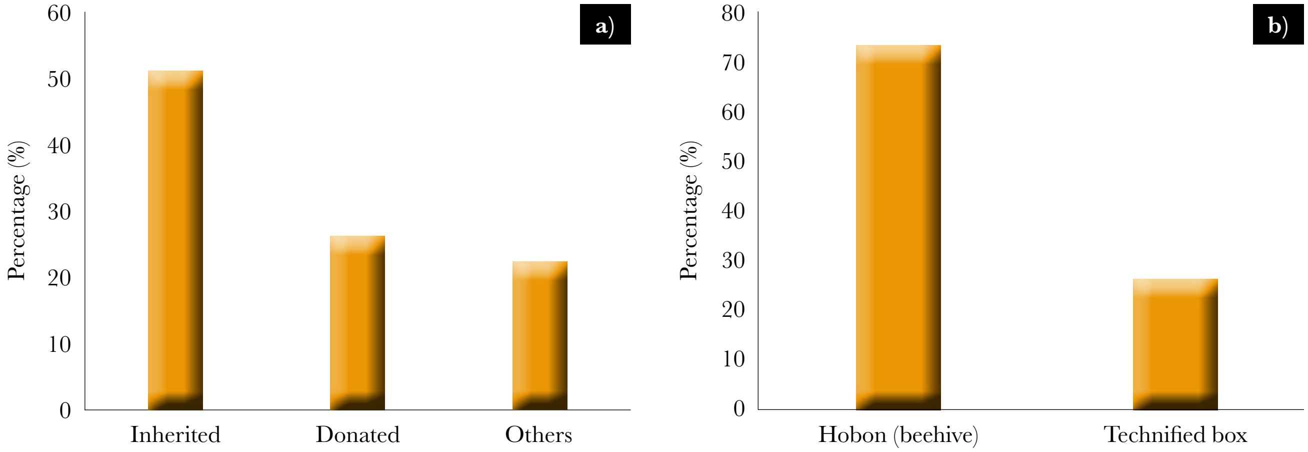

Figure 1. a) Origen of colonies, b) Nest type.

Biosphere Reserve (RBLP) in the State of Campeche underwent major changes in the past when several families abandoned the trade. The same authors argue that some of the main causes for quitting bee farming are the sale of colonies, poor management, drought, floods, among others.

Main characteristics of meliponaries

Modern meliponaries use galvanized metal sheets for roofing, instead of the old huano leaves. They also work with technified boxes rather than hollowed-out trunks to breed native bees (Table 1; Figure 2a; 2b).

These modern implements offer better conditions for handling bees and harvesting honey. They also reduce the possibility of contamination compared to the traditional harvesting process (personal observation). This new management system, based on the knowledge of the species' biology, can increase honey production and thus improve the meliponiculturists' income (Enriquez *et al.*, 2005).

Main characteristics of hobones

The average length of *hobones* (n=39) used in the studied localities is 59.13 cm, with a minimum of 45 cm and a maximum of 84 cm. The average height of each hobón is

Table 1. Construction of meliponaries, main characteristics.

Meliponary	Ceiling	Floor	Base type for colonies	Type of accommodation of the colonies	Meliponary location	Meliponary measurements (m)
1	Huano (palm)	Cement	One colony upon another	Hobon (beehive)	Backyard	4.5×3.0
2	Huano (palm)	Cement	Wooden shelf	Technified box	Backyard	3.0×3.0
3	Galvanized	Dirt floor	One colony upon another	Hobon (beehive)	Outside the house, but inside the community	7.0×3.0
4	Cardboard roofs	Dirt floor	One colony upon another	Hobon (beehive)	Backyard	1.5×1.0

Figure 2. a) Traditional meliponary withh huano roof. b) Meliponary with galvanized metal sheet roof.

27.62 cm, with a minimum of 23 cm and a maximum of 40 cm. They are 3 cm thick. The main wood species used to make *hobones* are cedar (*Cedrela odorata*), chicozapote (*Malnikara zapota*), yaxnik (*Vitex gaumeri*), tzalam (*Lysiloma bahamensis*), and jabín (*Pisidia piscipula*) (Figure 3).

In contrast, technified boxes (Figure 4) are specifically made to facilitate management practices and honey collection without damaging the hive, without wasting product, and, most importantly, in a clean and hygienic way (The Nature Conservancy, n.d.). The advantage of using technified boxes to breed stingless bees is that they can be opened to assess the state of the hives more easily, thus facilitating the honey extraction process without damaging the nest. They also allow for making divisions more comfortably (Enriquez *et al.*, 2005).

Honey harvest technique and production volume

There are two techniques for harvesting honey: the traditional and the technical method. The former consists in removing the clay from one end of the *hobón* and then perforating the honey pots, letting the honey drain over a bucket. A tulle cloth placed over the opening of the bucket serves to filter the honey and remove the clay remains. The technical method

Figure 3. Traditional hobones.

Figure 4. Technified box.

consists in piercing the honey pots with a needle and collecting the honey using a syringe (Figure 5).

Half of the producers harvest honey using the traditional method, while the other half uses the technical one (Figure 6). On average, 0.48 L of honey is collected per colony per year, with a yield of honey per colony per year of 0.50 L in technified boxes and 0.44 L in hobones.

Colony division and artificial feeding

Artificial feeding is a new technology that has spread among meliponiculturists. It consists in feeding the melipona bee colonies with honey of the *Apis mellifera* species (25% of producers implement it). Another practice is not harvesting the melipona colony if the producer does not deem it populated enough.

Sale price and destination of *M. beecheii* honey

The average price for a liter of melipona honey is 850 pesos, with a minimum of 750 and a maximum of 1,000 pesos. Honey is harvested twice a year: first between April and June and then between October and November.

Figure 5. Honey extraction using syringes.

Figure 6. Traditional technique of honey extraction.

Of all the harvested honey, 89.8% is destined for sale and 10.2% for self-consumption. Among the interviewed meliponicultorists, only one registers his activities (in a notebook). One of them transforms honey into other products, mainly soap, which they offer at a price of 60 pesos and sell one unit per month. In addition to selling honey, beekeepers can use melipona bees in greenhouse agriculture production, since bumblebees (Tribe: Bombini) are usually used for pollination (Quezada, 2005; 2009).

Main uses of *M. beecheii* honey

All interviewed meliponiculturists use melipona honey to treat cough and respiratory diseases. They claim to have acquired this knowledge through a relative, mainly the producer's mother or grandfather. Only one of them (25%) uses honey to treat cataracts, something they learned from their grandparents. Meanwhile, three of them (75%) use honey in some Maya ceremonies, mainly first fruits, which are celebrated in April to express gratitude for the first honey harvest. This evidence confirms that using honey in ceremonies is still a common practice in these localities. According to Sánchez-Aroche (2016), a tradition practiced in the States of Yucatán and Quintana Roo is the preparation and consumption of balche' (a drink made from honey and bark from the *Lonchocarpus longistilus* tree). This drink is used in rituals to sanctify and purify both sacred spaces and altars. Among these rituals are Ch'a' Chaak, Alborada (Dawn) or Xchok, Hoch Che', and U Hanli Chaco'ob. However, its production and consumption are kept secret from external agents. It has also been used to treat "ill wind" and a very sore body.

CONCLUSIONS

Although the number of people who practice meliponiculture has decreased, this activity still prevails in Campeche. People still use melipona honey for health purposes. In addition, this type of honey plays an important role in religious customs. Meliponiculture in Campeche is rooted in rural communities and still retains its economic, cultural, and social significance.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Palatability of animal oils included in the diet of the Mexican axolotl and its effect on growth and survival

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ABSTRACT

Objective: The growth, survival, and condition factor of *Ambystoma mexicanum* juveniles fed with three commercial feed-based diets coated with different animal oils as potential palatability enhancers were evaluated.
Methodology: Three diets were prepared using commercial feed coated with fish, chicken, and krill oil. The experiment lasted 81 days, the food was provided to the axolotls (6 per experimental unit, in triplicate) every 48 h, at 4% of the total biomass. The total weight of each experimental unit was recorded every 15 days, at the end of the experiment individual weight and height and survival were recorded. The following variables were calculated: Fulton's K, specific growth rate, coefficient of variation of final weight and size heterogeneity.
Results: All three treatments showed an increase in total biomass, with a trend towards better performance in chicken and fish oil treatments compared to krill oil. When performing the statistical analysis, it was found that there were no significant differences among the treatments for any of the variables recorded.
Implications: The three oils used in the present study were good palatable agents in the food intake of *A. mexicanum*.
Conclusions: It is recommended to use chicken oil as an attractant additive in the formulation of a specific diet due to its low cost.

Keywords: *Ambystoma mexicanum*, amphibian, nutrition, feeding, development.

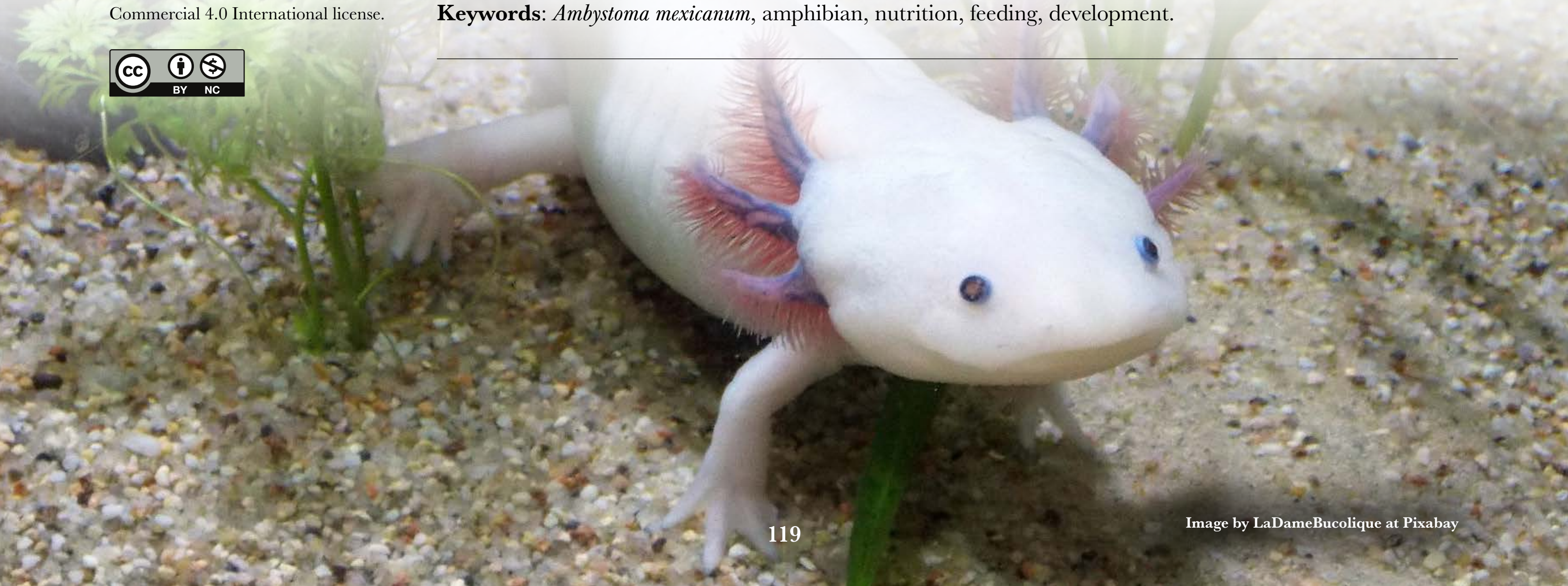

Image by LaDameBucolique at Pixabay

INTRODUCTION

Due to its biological characteristics, such as its regeneration capacity, one of the species that is used as a biological model around the world is *Ambystoma mexicanum* (Shaw & Nodder, 1798), endemic species of Lake Xochimilco, Mexico, which is in danger of extinction due to pollution and the introduction of invasive species that have reduced its populations, in its natural habitat. In contrast, its reproduction in captivity has favored its conservation and wide distribution in the world, either as a biological model or as a pet (Gresens, 2004; Vance, 2017; Gonzalez & Zamora, 2014). One of the biggest challenges of axolotls in captivity is feeding. Especially in its early stages as its diet is mainly live food (zooplankton) due to the size of its mouth being very small (Chaparro-Herrera *et al.*, 2011). When they reach a size of 5 cm in length they can begin to consume pelleted feed, although this transition is difficult as these diets are not very palatable for the animals, so it is recommended to use feed with ingredients that are attractive and palatable (Gresens, 2004). Currently there is no specific food for *A. mexicanum*. There are commercial products that are theoretically designed for the species, but they are not supported by scientific publications or by patents that prove it. In addition, they are difficult to acquire, so commercial fish feed is used conventionally (Gresens, 2004), with the logical nutritional deficiencies by not satisfying the specific requirements for the Mexican axolotl.

Axolotls, being aquatic, base their feeding strategy mainly on smell and taste, so it is important to find ingredients that are attractive and palatable for their food (Farkas & Monaghan, 2015). The palatability of a food is influenced by the ingredients present in it, which stimulate taste and intake. Likewise, the ideal is that these palatable ingredients also provide the nutritional requirements of the species to which the food is directed (Tantikitti, 2014).

Protein ingredients of marine origin have a greater number of low weight molecules, soluble in water, which give them high attractability, (for example fish and krill meal), compared to ingredients of terrestrial origin, so the latter are less used in the production of balanced feed for aquatic animals (Villarreal-Cavazos *et al.*, 2017); however, little has been studied on the effect of oils, insoluble in water, on the palatability of food, in captive axolotls.

The objective of the present study was to determine the palatable capacity of different animal oils added to the food of *A. mexicanum* and their effect on growth and survival, with the intention of designing better specific diets for the culture of the species in captivity.

MATERIALS AND METHODS

Obtaining of the axolotls

The juveniles of *A. mexicanum* were donated by the production center AXOS-PIMVS localizado en Tepic, Nayarit (Bahía de Banderas 62, Lomas de la Cruz, 63037). The specimens were transported in individual bags from the production center to the Laboratory of Water Quality and Experimental Aquaculture (LACUIC), belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco (20° 40"N; 105° 16"O / 20.667, −105.267). After an acclimatization period of 30 minutes they were placed in a 150 L tank in the laboratory with a controlled environment at a stable temperature of 18±1° C as recommended by Farkas and Monaghan (2015).

Experimental design

For the feeding bioassays, 6 axolotls (29.67±8.20 g) were randomly placed in 80 L tubs (gauged at 35 L) per treatment and in triplicate (each tub represented one experimental unit). 100% water exchanges were carried out every 48 h. Prior to the experiment, the weight and initial size of the axolotls were analyzed with an ANOVA ($P<0.05$).

Diets and food

Diets were prepared using commercial Growfish® brand pelleted feed for developing Tilapia, stage 2 (3.5 mm) (35.0% min. protein, 3.5% min. fat, 5.0% max. ash). The alternative diets evaluated consisted of the commercial feed coated with: krill oil (Simi Krill®) and chicken oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®). To avoid differences in lipid levels between these diets and the control, the latter was coated with fish oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®).

1020 g of feed were prepared for each treatment at 3% weight/weight inclusion of the oil (30 g of oil in 990 g of feed). The 990 g of food were placed in trays in an oven at 60 °C for 30 min (in order to remove moisture from the feed and make it better absorb the oil), then it was removed from the oven and placed in airtight plastic bags, for cooling and storage (24 h at 18±1 °C, in a dry room), until use. The feed was placed in a commercial mixer (Kitchen Aid®) to keep it moving while it was sprayed with each attractant oil using the spray method proposed by Oikawa & March (1997) and Kolkovsky *et al.* (2000) using undenatured ethyl alcohol to dilute the oil and make it easier to apply. Once the feed was coated, it was left to dry on a tray at room temperature for 15 min, after which it was placed for 15 min at 60 °C in an oven and finally it was left to dry for 24 h at 18±1 °C to ensure evaporation of the alcohol. After preparing the diets to be tested, proximate analyzes were performed to ensure the levels of proteins, lipids and ash of the treatments were similar between the diets.

The experiment lasted 81 days during which the food was provided to the axolotls every 48 h, at 4% of the total biomass, and the unconsumed food was withdrawn 24 h after being administered. The total weight of the animals in each experimental unit was recorded every 15 days and at the end of the experiment (81 days) a biometry was performed where individual weight and size of each organism were recorded, as well as survival. With the data obtained, the following variables were calculated: Fulton's $K\left(K=\left(W/L^{3}\right)\times 100\right)$, specific growth rate $\left(SGR\%=\left[\left(\ln W_{f}-\ln W_{i}\right)/t\right]\times 100\right)$, coefficient of variation (CV) of the final weight (Kestemont *et al.*, 2003) and size heterogeneity $\left(\text{heterogeneity of sizes}=CV_{wf}/CV_{wi}\right)$; where W_f=final weight (g), W_i=initial weight (g), t=time (days) and CV=coefficient of variation.

To analyze the level of conditioning to the test diets, the food consumed was quantified at the end of the 81 days of the experiment. The axolotls were fed (at 4% of their biomass per experimental unit) once more and the residual food was removed after 24 hours.

Immediately afterwards, it was dried in an oven at 60 °C for 24 hours, placed in airtight plastic bags for cooling and storage (24 h at 18±1 °C, in a dry room), and its weight was recorded.

Statistical análisis

With the data obtained, the Kolmogorov-Smirnov test was performed to verify that the data presented normality and homoscedasticity. After that, an (ANOVA) was performed. In cases where significant differences were found between treatments ($P<0.05$), Tukey's test was performed for comparison. These analyzes were performed using SigmaPlot11 software.

RESULTS AND DISCUSSION

Figure 1 shows the biomass increase of axolotls over 81 days of experimentation using diets with different types of attractants (fish oil, chicken oil and krill oil). All three treatments showed an increase in total biomass, with a trend toward better performance in chicken and fish oil treatments compared to krill oil. However, when performing the statistical analysis, it was found that there were no significant differences between the treatments for any of the variables recorded (Table 1).

The similar consumption of the different diets among the three treatments could be due to the fact that the palatability stimulus caused by the different oils was similar for the axolotls. Which suggests that chicken oil, despite being terrestrial, worked in a similar way to marine oils, which are more expensive. These results coincide with those reported by Sanches-Alves *et al.* (2019) for *Oreochromis niloticus* where hydrolyzed poultry protein was the one that induced a higher feed intake for this species compared to other ingredients, including fishmeal and pork liver.

Farkas & Monaghan (2015) reported that smell is an important sense for feeding in axolotls because they lack an auditory median structure and their vision is very poor and limited to motion detection. But this last characteristic is another reason that could explain the similarity of the results of food consumption, in the present study since the axolotls, when observing the movement of the food at the moment of sinking, reacted quickly

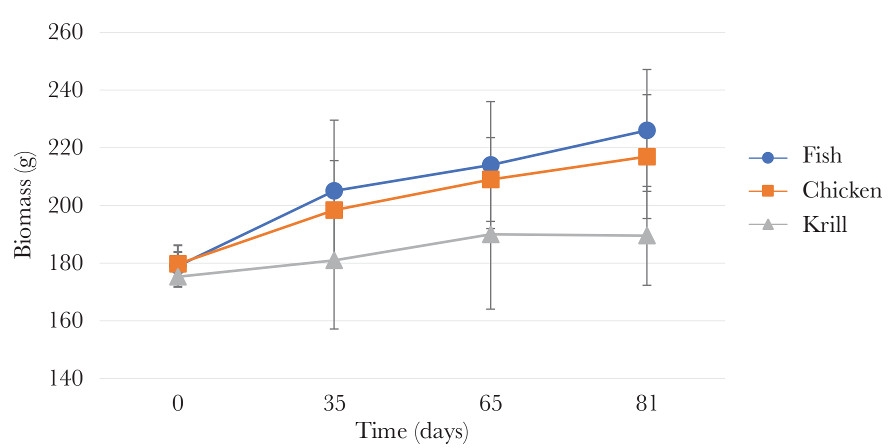

Figure 1. Increase of the total biomass in *A. mexicanum* with different types of attractants added to the diet.

Table 1. Weight, length, survival, size heterogeneity, weight gained per day, Fulton's K, specific growth rate (SGR) and food consumed (mean ±S.D. of three replicates per treatment) in *A. mexicanum* fed three diets for 81 days including different animal oils as attractants.

Diets	Fish	Chicken	Krill
Survival (%)	100±0.00	94.44±9.62	88.89±9.62
Initial weight (g)	29.83±1.20	29.94±0.70	29.22±0.34
Final weight (g)	37.70±3.52	38.38±2.92	35.60±1.34
Coefficient of variation (final weight g)	15.97±2.93	17.85±0.36	18.60±3.95
Size heterogeneity (g)	0.63±0.08	0.61±0.20	0.58±0.16
Initial lenght (cm)	15.19±0.13	15.08±0.14	15.04±0.29
Final lenght (cm)	15.97±0.34	16.20±0.48	16.22±0.38
K- factor	0.92±0.04	0.90±0.03	0.84±0.08
SGR (%)	0.28±0.07	0.30±0.07	0.24±0.05
Weight gained per day (g)	0.10±0.03	0.10±0.03	0.08±0.02
Food consumed (g)	2.60±0.54	2.53±0.52	2.27±0.48

No superscripts were added as no statistically significant differences were found (one-way ANOVA, $P<0.05$).

and indistinctly to capture and ingest it. No rejections due to regurgitation of food were observed in any of the treatments.

Although there were no significant differences between the treatments, a trend was observed that suggests a potential rejection (lower palatability compared to chicken and fish oil) to krill oil, with lower intake results, with negative effects on survival and final weight, compared to chicken and fish oils. The apparent better palatability and intake of feed coated with chicken oil represents an economic advantage in the formulation of a feed since it would not be necessary to add a high-cost oil such as krill oil to the diet of axolotls to obtain a good performance. According to Lee & Meyers (1996) and Nunes *et al.* (2006), the food ingredients obtained from aquatic animals (flours and soluble fractions) have low weight molecules that make them excellent attractants (Smith *et al.*, 2005; Ali *et al.*, 2007), while those obtained from terrestrial animals (oils and poultry meals) do not have this quality (Suresh *et al.*, 2011). Although there are studies in other species that favor the use of krill as an attractant (Oikawa & March, 1997; Kolkovski *et al.*, 2000) in the present work it was not demonstrated it is a more palatable ingredient of primary choice for axolotls since chicken oil obtained better results; chicken oil obtained better results; which coincides with what was reported by Suresh *et al.* (2011) in marine shrimp. In fact, in the only study carried out with the genus *Ambystoma* and specifically with *Ambystoma mexicanum*, by Ocaranza-Joya *et al.* (2021) found no differences in the response of animals to the attractant/palatable effect of krill and chicken oils, concluding that both can be used effectively for this purpose.

The condition index (Fulton's K) showed that the axolotls presented good physical condition between the three treatments, they did not present erratic swimming or diseases after the experiment. The values presented in this study were better than those reported for larvae of the same species ($K=0.79$) in studies that evaluated the development and growth of larvae in three different maintenance media (dechlorinated tap water and enriched with sodium chloride; commercial colloidal solution; Holtfreter's solution (Robles-Mendoza *et al*., 2009). In relation to survival, these authors report 10% mortality) which is similar to what was observed in this study, since survival was greater than 90% in all treatments.

Although a low heterogeneity of sizes such as the one presented in this study, by itself is not considered an index of condition, in the culture of aquatic animals it is sought that the production be as similar as possible. Especially for cannibal species such as axolotls since the reduction of differences in sizes can reduce type II cannibalism (complete cannibalism) as indicated by Baras *et al*. (2000) and Kestemont *et al*. (2003).

Maya-Monroy (2006) carried out an experiment with *A. mexicanum* larvae with three different diets for a year: a diet with commercial food for turtles ("Tortuguetas"®), a diet based on insects (crickets) and a mixed diet of the previous two. The author found significant differences between these three diets, being the insect-based diet the one that presented the best result in weight and length of the axolotls. The author mentions that despite the fact that the turtle feed has a higher amount of protein per kilo (300 g) compared to a kilo of crickets (205 g), the former was not well accepted by the axolotls, and was even rejected when it was administered to the animals directly in the mouth. This corroborates the importance of developing a palatable and attractive food for the species, which was the main objective of this study.

In that same study (Maya-Monroy, 2006) reports that at the end of the 12 months of experiment the growth curve of the axolotls reached a type of asymptote, where it is slower, unlike the first months of the experiment. This could explain the results of the present study, where the difference between the initial length and final length was not very notable, since it is probable that the animals used in the present study could have been late juveniles in which the exponential growth stage was already had happened.

CONCLUSIONS

In conclusion, the three oils used in the present study were good attractants for the food intake of *A. mexicanum*. However, it is recommended to use chicken oil as a palatable additive in the formulation of a species-specific diet due to its low cost. According to these results, it is suggested to carry out nutritional experiments where different levels of inclusion of chicken oil in the diet are evaluated to determine the best concentration for the species. This study provides knowledge to improve the captive maintenance of this endangered species.

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AP AGRO PRODUCTIVIDAD

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Avocado (*Persea americana* Mill.) production in Huatusco, Veracruz, Mexico

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ABSTRACT

Objective: To characterize the avocado production system in Huatusco, Veracruz.

Design/Methodology/Approach: Interviews based on the snowball method were applied to avocado producers in Huatusco, Veracruz. Meanwhile, fruit and water samples were used to perform a multiresidue analysis of pesticides.

Results: The monoculture plantations are rainfed. They have a density of 100-150 trees ha^{-1}. The Hass variety predominates in Andosols, with 5-100% slopes. Most of the producers (92%) carry out conventional management, applying pesticides and chemical fertilizers. Herbicides and brushcutters are used to control weeds. No pesticides were detected in the water samples, while Imidacloprid, Thiabendazole, and alpha-Cypermethrin were identified in a fruit sample, with 0.0038±0.0014 mg/kg, 0.0022±0.0009 mg/kg and 0.0703±0.000 mg/kg concentrations, respectively. These concentrations fall within the limits allowed in Mexico.

Study Limitations/Implications: A detailed and frequent sampling of avocado fruits should be carried out to determine pesticide residues. It is also necessary to analyze samples of running water near the orchards to prevent pollution.

Findings/Conclusions: The empirical knowledge of the producers about the type of soil is more precise than the scientific classification: they identify five classes of soils beyond Andosols. Additionally, there is a marked inequality in land tenure, since large-scale producers (25% of all producers) own 86% of the established area. Although, in a preliminary sampling, pesticide concentrations did not exceed the limits of residues allowed in the avocado pulp, the number of pesticide applications should be reduced.

Keywords: *Persea americana*, Andosols, rainfed agriculture, conventional agriculture, pesticides.

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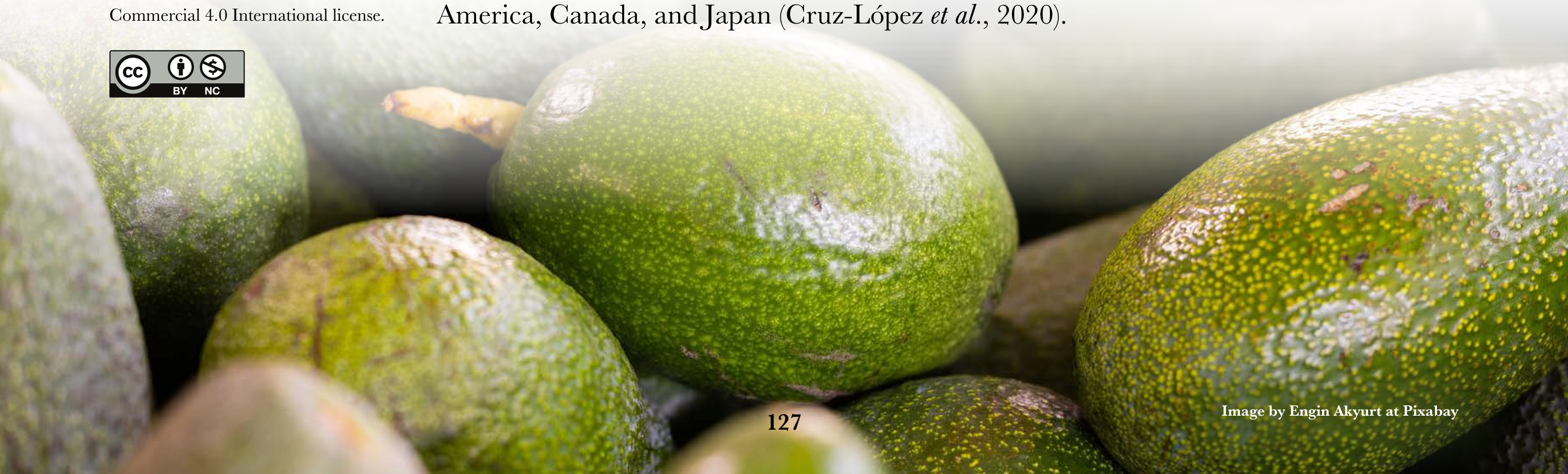
INTRODUCTION

There is a great worldwide demand for avocado (*Persea americana* Mill.) and Mexico is the main producer and exporter. In 2019, 722,000 t were exported, which represents more than 45% of the world total. The most important markets are: the United States of America, Canada, and Japan (Cruz-López *et al.*, 2020).



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In Veracruz, the producers do not only grow avocados: they carry out this activity to obtain additional income. They believe that their orchards are low-tech, as a result of a potential low soil fertility (Nataren-Velazquez *et al.*, 2020). Nineteen municipalities produce 7,393 t of avocado in 897 ha. Altotonga, Coscomatepec, Jalacingo, Atzalan, and Huatusco de Chicuellar (Huatusco) have the largest planted area. In Huatusco, 702 t are produced in 84 ha (SIAP, 2020); its orchards are located in a tropical high-altitude area where coffee is grown, while pear, plum, cherimoya, and kiwi are grown at a higher altitude (Guerra-Ramírez *et al.*, 2021).

The growing demand for avocado puts great pressure on the forests where it is cultivated, whose altitude ranges from 1,300 to 2,400 m.a.s.l. (Marroquín-Páramo *et al.*, 2017). The expansion and intensification of its cultivation results in a high potential for environmental impacts, such as the loss of native oak and pine forests, the reduction in carbon sequestration, the biodiversity loss (Denvir *et al.*, 2021), a change in land-use, and excessive water use (González-Estudillo *et al.*, 2017).

This growth also has social and economic impacts. Smallholder farmers, farm workers, and local communities receive a minimal share of the profits and suffer loss of their livelihood, governance, and community cohesion, as well as public health problems (De la Vega-Rivera & Merino-Pérez, 2021). Avocado production requires a fair treatment of workers and the sustainable use of natural resources.

In the future, avocado will be grown at higher and lower elevations, with less rainfall and in less suitable soil groups, which may lead to an increased application of fertilizers, pesticides, and irrigation (Franco-Sánchez *et al.*, 2018; Arima *et al.*, 2022). In the State of Mexico, avocado producers require technology to control pests, weeds, and diseases (Sangerman-Jarquín *et al.*, 2014), while, in Michoacán, water scarcity and the chemical contamination of groundwater are already problematic (Borrego & Allende, 2021). However, there is no information in this regard for Veracruz.

During the last 14 years, avocado has been introduced into a humid zone in Huatusco and its surrounding area, characterized by Andosols —a kind of soil that is susceptible to erosion, after the forest cover has been removed. The cultivation and production management of avocado has not been documented. The objective of this study was to agronomically characterize the avocado production system in the municipality of Huatusco, Veracruz.

MATERIALS AND METHODS

The localities studied in the municipality of Huatusco in 2021 were Elotepec, San Diego Tetitlán, and Huatusco (Figure 1).

Interviewees selection

We interviewed avocado producers in the municipality of Huatusco, Veracruz, using the snowball method —in which one person suggests another based on their knowledge and experience (Davis *et al.*, 2017). The sample size was determined by the point of informational redundancy and data saturation (Letts *et al.*, 2007). All the interviews (12) were semi-structured (Lazos-Ruíz *et al.*, 2016). The producers were classified according to

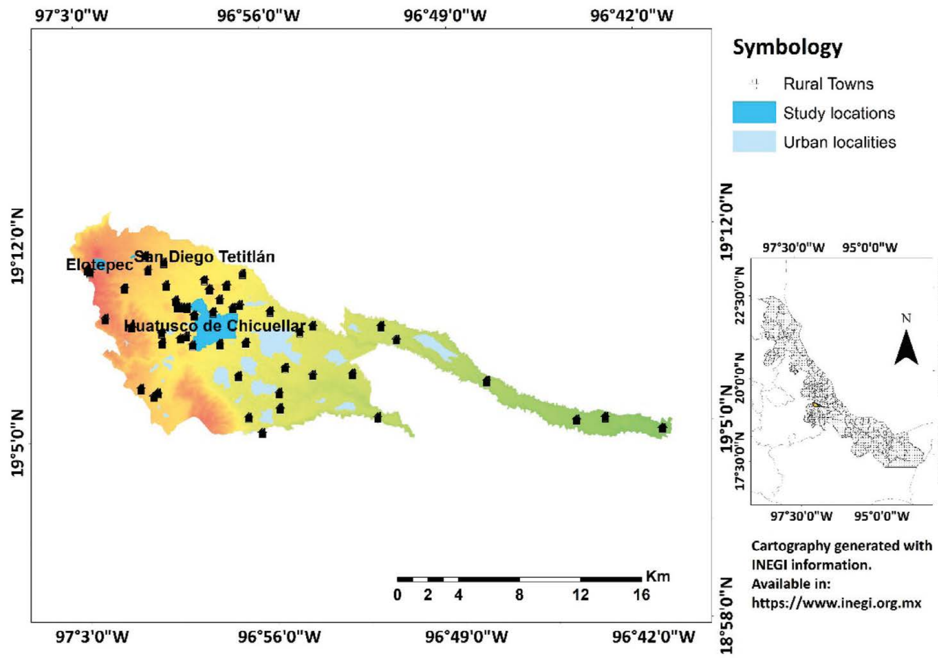

Figure 1. Study locations in Huatusco, Veracruz, Mexico.

the operating rules (2021) of the Producción para el Bienestar program of the Secretaría de Agricultura y Desarrollo Rural (SADER, 2020).

Analysis of pesticide residues in the fruit pulp and in the water

Water samples were collected from four representative sampling sites: two in a spring and two in surface streams that cross the avocado plots. In addition, two samples of locally produced avocado fruit were taken and the multiresidue pesticides were determined with QuEChERS extraction, by liquid chromatography tandem mass spectrometry (LC–MS/MS) (Aghris *et al.*, 2022).

RESULTS AND DISCUSSION

Among the studied localities (Figure 1), there was an altitudinal contrast and different soil classes, forms of management, and strata of producers. The producers interviewed indicated a diversity of characteristics related to the production system (Table 1).

Land tenure

Land tenure belonged to the private property system. Fifty percent of the interviewees were small-scale owners, 25% were medium-scale owners, and the remaining 25% were large-scale owners (SADER, 2020) (Table 1). Out of the total 243.85 ha, 209 were distributed among large-scale producers, 24 ha among medium-scale producers, and 10.85 ha among small-scale ones. In the State of Mexico, 33% of the producers concentrate 67% of the surface, while 82% only owns 4%, which proves the great inequality in land tenure and income (Sangerman-Jarquín *et al.*, 2014).

Table 1. Particularities of avocado orchards in Huatusco, Veracruz, Mexico.

Producer type	Property	Area (ha)	Slope (%)	Age	Management	Elevation (m)
Big scale	La Represa	100	10-100	1, 11, 13	Conventional	1729
Medium scale	El Nogal	9	10-100	13	Conventional	1771
Medium scale	El Cafetal	10	5-100	5, 7	Conventional	1396
Medium scale	Ahuacapa	5	20-60	4	Conventional	1351
Small scale	Huerto San Carlos	3.5	20-60	3, 7, 8	Conventional	1362
					Semiorganic (1 ha)	1362
Big scale	La Selva	24	15-60	10	Conventional	1405
Big scale	Finca Pastoría	85	15-60	3 (35 ha), 4	Conventional	1408
Small scale	El Encino	0.2	10-60	2	Organic	1817
Small scale	Centro Elotepec	0.15	20-60	10	Conventional	1962
Small scale	El Plan	1	20-60	2	Conventional	1856
Small scale	La Raya	2	20-45	10	Conventional	1834
Small scale	Xometla	4	50-100	4, 5	Conventional	1872

In Huatusco, 84 ha of avocado were reported in 2020 (SIAP, 2020), while a 290% growth in the avocado surface was reported the following year. Several orchards were established in areas that had primary vegetation, but the negative effects on biodiversity are unknown.

Age of the plantation and slope of the plots

The oldest plantations are 13 years old, while the youngest are one year old (Table 1). By the end of 2022, Finca Pastoría, in Felipe Reyes, was expected to increase its area by 30 ha.

The slopes (Table 1) ranged from 5% (steep) to 100% (very steep) (FAO, 2009). Soil erosion and runoff rates from hillside avocado orchards are not sustainable under current cultivation practices, where ground cover is suppressed (Atucha *et al.*, 2013).

Altitude of the plantations

Hass avocado plantations in Michoacán are located between 1,600 and 2,200 m.a.s.l. (Anguiano *et al.*, 2007), while in Huatusco they are located between 1,351 and 1,872 m.a.s.l. (Table 1). According to the producers, this is an adequate altitude and they recommend establishing new plantations between 1,200 and 3,000 m. Frost and hailstorms limit avocado cultivation in the region, mainly above a 1,700 m altitude. Hail is responsible for up to 90% of the losses of small fruit. Some orchards have lost up to 50% of their total production and anti-hail rockets have been used to counteract the problem.

Type of production system

Most producers (92%) perform conventional management, applying pesticides and chemical fertilizers (Table 1). One small-scale producer practices organic management and another is trying out a semi-organic system to reduce the use of chemical products.

The interviewees indicated that organic avocado production reduces damage to the environment.

One orchard uses a coffee agroforestry system, while the rest of the plots are monocultures with Fuerte, Hass, Hass-Méndez, Méndez, and Pinkerton varieties. Certified plants from Michoacan were established. Two producers used non-certified plants from Veracruz and Puebla. Native avocados and avocado-like species, such as *Persea schiedeana* (Cruz-Castillo *et al*., 2017), are not produced in commercial orchards in the study region. In Temascaltepec and Coatepec de Harinas, State of Mexico, Hass avocado production predominates (97%), while native avocado is cultivated to a lesser extent (3%) (Sangerman-Jarquín *et al*., 2014).

Plantation establishment

Eighty-three percent of the producers established their trees using a square system, varying their separation from 8 to 10m (100-150 trees/ha). In one orchard, the producers used a quincunx system, while another orchard has a contour system with 7 m between plants and 9 m between rows. The holes for the stumps were either 30×30×30 cm or 60×80×80 cm (depth×width×length). Twenty-five percent of the plots were filled with vermicompost, rooting agents, lime or beneficial fungi, and in one plantation recycled wire mesh was used to control rodents. In the municipalities of Coscomatepec, Calcahualco, and Alpatlahuac, Veracruz, the density ranges from 125 to 180 trees/ha (Nataren-Velazquez *et al*., 2020).

Soil, fertilization, and irrigation

The best soils for avocado cultivation have a pH of 5.5 to 6.5. They are sandy loam, well-drained, loose, and deep soils that guarantee good root development (Amórtegui-Ferro *et al*., 2001). As a result of their good permeability, the suitable soil groups for the establishment of avocado plantations are Andosols and Luvisols (Anguiano *et al*., 2007). All the plantations were established on Andosols (INEGI, 2019) and the producers know that the soils must be drainable, with an ideal pH of 5.7 to 6.0. In addition, they have identified a great diversity of soil classes, which they known as ***polvilla***, ***barial***, ***sámago***, ***colorada***, and ***barrialillo***. Producers associate black soil with good fertility.

Prior to fertilization, producers carry out a soil analysis in 50% of the plots, in two out of which they also consider the analysis of fruit, root, and leaf. This procedure is associated with medium- and large-scale producers. They mainly apply: diammonium phosphate, potassium nitrate, calcium nitrate, magnesium sulfate, calcium superphosphate, and elemental sulfur. In large-scale orchards, the pH of the water used to apply fertilizers and pesticides is regulated. In 25% of the plots, they also add vermicompost, fulvic and humic acids, coffee pulp, and manure. In the only organic orchard, producers use manure to supplement nutrients.

In some plots, fertilizers are applied without using soil analysis. As a result, fewer nutrients are applied to maximize production or an excessive application leads to nutritional imbalances that limit production give rise to environmental problems (Shunfeng *et al*., 2018).

Irrigation is non-existent and all the plantations are rainfed. Producers indicate that a tree requires 40 to 70 liters of water per week and that the average annual rainfall exceeds 1,000 mm. The Hass variety requires between 1,200 and 1,800 mm of annual rainfall (Bartoli, 2008). The avocado water footprint ranges from 1,981 m^3/t (Mekonnen & Hoekstra, 2020) to 4,945 m^3/t (Reyes-Pineda & Naranjo, 2021). The relevant information for the study region is non-existent.

Pruning

In all orchards, sprout pruning is carried out when the trees are 0.5 to 2 years old. Subsequently, the canopy formation is pruned, leaving 3 to 4 lateral branches. In two orchards, producers perform clearance pruning, which improves aeration and light entry (Huaraca *et al.*, 2016). In some orchards, the size of the trees has been reduced by eliminating their central axis (Viteri *et al.*, 2021). This practice is important, as the excessive humidity in Huatusco promotes vigorous growth in avocados.

Pests and diseases control

Stem, seed, and fruit borers, thrips, red spider mites, whiteflies, and white grubs are controlled with malathion, imidacloprid, dimethoate, abamectin, naled, and dimethyl. Two orchards applied soybean or lemon oil, mineral salt, garlic and chili-based products, resins, cucumber extract, and *Beauveria bassiana* for biological control. A couple of producers mentioned performing manual control of the stem borer. The avocado seed borer (*Heilipus lauri*) has been reported in Huatusco (Castañeda-Vildózola *et al.*, 2009). In Acaxochitlán, Hidalgo, Ortega-Licona *et al.* (2016) reported that four borers attacked the avocado fruit: *Heilipus lauri*, *Conotrachelus perseae*, *Cryptaspasma perseana*, and an unidentified Tortricidae species. The use of geostatics-based spatial distribution could help avocado growers in Huatusco. In Coatepec de Harinas, State of Mexico, producers carried out biological control of *Trips* spp. using *Amblyseius swirskii* (Acosta-Guadarrama *et al.*, 2017). This methodology has also been used to control the red spider mite (*Oligonychus punicae* Hirst) in two municipalities of the State of Mexico; this has resulted in the optimization of the use of agricultural inputs, causing less environmental damage (Lara-Vázquez *et al.*, 2018).

Regarding diseases, the producers mention that the fruit is attacked by clavibacter, anthracnose, scab, and black spot, which are controlled with calcium sulfide solution, copper sulfate pentahydrate, pyraclostrobin, azoxystrobin, fludioxonil, metiram, thiabendazole, quintozene, and thiram. During flowering and fruit mooring, they use hydrated copper to prevent fruit drop. Unlike the case of Huatusco, other places use the fungicides azoxystrobin + fludioxonil (Gonzalez *et al.*, 2020) to control *Colletotrichum* spp. and *Lasiodiplodia theobromae*, which cause anthracnose and peduncular rot, respectively. Diseases are frequent, as a result of a high relative humidity (70-85%) and temperatures of 20 °C or more. This situation favors an excessive application of pesticides to combat diseases. In the State of Mexico, anthracnose causes economic losses that can reach up to 20% of the production (Tapia-Rodríguez *et al.*, 2020).

Weed control

The producers control weeds by hand using a brushcutter or machete and they use glyphosate in 25% of the orchards. In one plot, they apply paraquat on the slopes and glyphosate on the level ground, arguing that this reduces erosion on the slopes. The producers did not mention either the use of cover crops to control weeds and incorporate plant residues or of microorganisms that improve soil quality (Huaraca *et al.*, 2016).

Analysis of multiresidue pesticides

In an environment with high temperatures and excess humidity, pesticide applications are numerous. Consequently, analyzing pesticide residues in fruits and running water is of the utmost importance. No pesticides were detected in water samples from spring and surface streams. In one of the avocado pulp samples, the presence of three pesticides —with values below the maximum residue limit allowed in Mexico (MRL)— was identified: Imidacloprid at 0.0038±0.0014 mg/kg (MRL of 1 mg/kg), Thiabendazole at 0.0022±0.0009 mg/kg (MRL 10 mg/kg), and alpha-Cypermethrin at 0.0703±0.000 mg/kg (which has no registered MRL for Mexico). A greater presence of pesticide residues in the pulp than in the peel can be the result of the lipophilic characteristics of the pulp is lipophilic and the analyte diffusion from the peel into the fruit. Likewise, analytes may leach during the harvest and the transportation process and some of them may enter the fruit (Betancourt-Arango *et al.*, 2021). This is the first time that pesticide residues have been documented in an avocado producing area of the state of Veracruz.

Harvest and postharvest handling

The dry matter of the fruit (22-24%) is considered to carry out the harvest. The fruits are also harvested when they become opaque or matte green. In the postharvest period, the fruits are refrigerated at 5 °C for 15 days, when they are sent to distant cities such as Monterrey. High dry matter values (*e.g.*, 26±2%) prevent weight loss, cold damage, and pulp damage after maturation (Escobar *et al.*, 2019).

Commercialization

The consumer market has a domestic scope and the produce is sent to self-service warehouses located in Orizaba, Huatusco, and Nogales, in Veracruz, and to the cities of Puebla and Monterrey. Small-scale producers sometimes sell their crops to large-scale producers or rent refrigeration facilities. They also point out that the small and medium-sized commercial ranges (120-150 g and 150-170 g, respectively) are sent to supply centers in Puebla. The yields mentioned by the producers are in a range of 3.2 to 10 t/ha. In 2020, the volume of avocado production in Mexico amounted to 2.4 million ton in a harvested area of 220,000 ha, which gives an average yield of 10.9 t/ha (STATISTA, 2020). In the south of the State of Mexico, the production is destined for domestic consumption: it is mainly marketed in the supply centers of Toluca, State of Mexico, and Mexico City, as well as in regional markets (Rubí-Arriaga *et al.*, 2013).

CONCLUSIONS

The empirical knowledge of the producers about some variables of the production system is similar to the data we found in scientific publications. Producers identify five types of soils (in addition to Andosols): *polvilla*, *barrial*, *sámago*, *colorada*, and *barrialillo*. Likewise, they associate black lands with good fertility. Avocado cultivation does not represent their main economic activity. There is inequality in land tenure, since large-scale producers (25%) own 86% of the established area. Most of the orchards use a monoculture system with the Hass varieties. Conventional management predominates in orchards with steep and very steep slopes. An excessive volume of pesticides and chemical fertilizers is applied. Glyphosate is one of the main herbicides used. In a preliminary study, no pesticide residues were found in running water near the orchards, and the pesticides found in the pulp are within the limits allowed in Mexico. The consumption market has a domestic scope and its yields have a range of 3.2 to 10 t/ha. The environmental impact and the effects on biodiversity need to be taken into consideration, because many of the orchards substituted primary vegetation.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Procurement and characterization of cellulose nanocrystals from cassava bagasse (*Manihot esculenta* Crantz)

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ABSTRACT

Objective: To procure and characterize cellulose nanocrystals from cassava bagasse.

Design/methodology/approach: Cellulose nanocrystals were obtained from cassava bagasse by acid hydrolysis (HCl), ultrasonication, centrifugation, dialysis, deep freezing and lyophilization. The cassava bagasse and the cellulose nanocrystals obtained were physicochemically characterized by Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy with Coupled Elemental Analysis (SEM-EDS). As an additional technique, Atomic Force Microscopy (AFM) was used.

Results: The analyses performed show that the cellulose obtained was type I′. This study reports a percentage of crystallinity of the cassava bagasse cellulose of 37.1%, increasing the percentage to 48% crystallinity in cellulose nanocrystals. The diameters of the cassava bagasse fibers were reported to be 2 μm and their elemental composition (SEM-EDS) mainly constituted by carbon (C), oxygen (O) and traces of nitrogen (N). The morphology observed through AFM of the nanocrystals of cassava bagasse (*Manihot esculenta*) was rod-shaped, with helicoidal appearance without residual charge, with diameters between 8.7 and 9.3 nm.

Limitations on study/implications: The acid hydrolysis process showed a low percentage of crystallinity, although higher than other works reported for cassava bagasse.

Findings/conclusions: The results obtained confirm the possibility of obtaining cellulose nanocrystals from cassava bagasse (*Manihot esculenta*).

Keywords: Cassava, cellulose nanocrystals, physicochemical characterization.

INTRODUCTION

Worldwide, approximately 60 million tons of starch are extracted annually derived from cereals, roots and tubers. Its uses vary widely, as stabilizing agent in soups and frozen foods, coating for pills and paper, adhesive for stickers and laminated wood, as a

Image by Fernando Augusto at Pixabay

finishing agent on textiles, raw material in the production of ethanol, and even as cohesion agent in concrete. About 10% of this starch is derived from cassava roots (FAO, 2006). Cassava root starch is the cheapest food source to be found in the world, and it is used in over 300 industrial products (Rivera-Hernández *et al.*, 2012). Its composition is made up of glucose particles that are divided into two types: amylose or simple glucose, and amylopectin, a branching form of glucose. This is the quality that makes starch such a good source of energy for the organism (Villalobos and Thorpe, 1984). The solid residues produced during starch extraction are: peels, damaged tips, bran, and residual starch stillage (Cereda, 2001). Cassava bran or bagasse (Sriroth *et al.*, 2000; Pandey *et al.*, 2000) is a semisolid fibrous material with a high moisture content, resulting from the process of starch granule separation during the sifting stage (Cereda, 1994). According to Breuninger *et al.* (2009), for every ton of starch processed, approximately 350 kg of cassava bagasse are obtained. However, the high moisture content in the bagasse makes storage and transport difficult, as well as causing leaching processes which can affect the environment, which is why it is advisable to identify an alternate way to use it (Marmolejo *et al.*, 2008). Cellulose nanocrystals (whiskers) are very thin monocrystals (1-100 nm) that offer many advantages as reinforcement/barrier particles in polymer matrix compounds (Dufresne, 2006). They possess a high degree of crystalline perfection, high rigidity (the traction resistance of a single cellulose crystal is theoretically estimated at between 0.3 and 22 GPa), and mechanical properties, so they have exceptionally elevated resistance. Additionally, they are derived from a renewable source and, being biodegradable, can potentially offer low environmental risks (Lahiji *et al.*, 2008). With the aim of contributing to the sustainable management of the environment, and tending to a better handling and incorporation of the residues into the productive cycle, by reusing or transforming them into other products with a high added value, the study proposes the exploitation of cassava bagasse for its transformation into cellulose nanocrystals.

MATERIALS AND METHODS

The cassava bagasse was obtained from the pilot plant at Universidad Popular de la Chontalpa for cellulose extraction; it was dried in an oven (Ecoshel® model HV-50) at 60 °C for 2 days, pulverized with the help of a mortar, and then stored in hermetically sealed bags.

Procurement of cellulose nanocrystals (CNC)

A 350 mL solution of HCl was mixed at 4N with 10 g of cassava bagasse, agitating continuously for 225 min at 60 °C, and distilled water was added at a 1:5 ratio (with the purpose of halting the reaction). Then an ultrasonication cycle was applied at 750 Watts (with 50% amplitude) during 3 min. The solution obtained was subjected to repeated washing with deionized water at a centrifuge speed of 10,200 rpm during 10 min, until pH=4 was reached (Araki *et al.*, 1998), in order to collect a cloudy whitish supernatant which was stored and then concentrated with the help of a BUCHI® brand rotary evaporator until a colloidal suspension was reached. The nanocellulose concentrate was purified through dialysis membranes (12-14 kD) during 5 days, until a pH equal to that of the deionized water used was reached. This suspension was deep frozen for 3 days at −18 °C for its subsequent lyophilization and storage.

Physicochemical characterization

To determine the functional groups of the cassava bagasse and of the CNC, a Nicolet Nexus 670 spectrometer was utilized, on absorbency mode, with a resolution of 4 cm^{-1} and 100 scans, using 1 mg sample pellets on 100 mg of KBr.

The crystallinity of the cassava bagasse and the CNC were determined via the powder X-ray diffraction method (PXRD), using a Bruker D-8 Advance diffractometer, in Bragg-Brentano geometry, exposure time of 0.5 sec, size of exposure 0.02 degrees, spectrum of CuK′ (α=1.5418 Å and 8.047 keV energy). The percentage of crystallinity of the cellulose obtained was calculated using the method described by Segal *et al.* (1959), using the equation (1).

$$X_C\% = 100\left[1-\left(\frac{I_1}{I_2}\right)\right] \qquad \text{Eq. (1)}$$

Where: I_1 is the minimum intensity of the crystalline peak and I_2 is the maximum intensity of the crystalline peak, respectively (taken from the diffractogram results generated during the XRD analysis).

In order to analyze the morphology of the cassava bagasse and perform its elemental analysis, a Phillips and XL-30 ESEM JEOL JSM-7600F scanning electron microscope with energy-dispersive spectroscopy (SEM-EDS) was utilized. The cellulose CNC were characterized with an NX10 Biometer atomic force microscope, combined with analysis of the images in order to determine the different sizes of particles, using an intermittent reading mode, scanning the surface areas on planes (X-Y).

RESULTS AND DISCUSSION

Chemical characterization of the functional groups via FTIR

Figure 1 (a and b) shows the interferograms (FTIR) of cassava bagasse (*Manihot esculenta*) and the cellulose nanocrystals, respectively.

In the spectra we observe peaks at certain frequencies that are characteristic of different groups: the wide peak that spans 3500-3200 cm^{-1} corresponds to the stretches of the OH group of the free and bonded hydroxyl, intra- and inter- molecularly present in the anhydrous glucose units of amylose and amylopectin (Enríquez *et al.*, 2010). On the other hand, both spectra demonstrate the characteristic C-H stretching vibration around 2900 cm^{-1} (Rosa *et al.*, 2012). A C=O bond was observed from 1730-1740 cm^{-1}, which is characteristic of lignin and hemicellulose (Abraham *et al.*, 2011). Figure 1a shows a peak at 1645 cm^{-1} which is associated with bending of the OH group of the adsorbed water (Alemdar and Sain, 2008; Abraham *et al.*, 2011), as well as bending curves in the C-OH plane on the peaks close to 1340 cm^{-1} corresponding to holocellulose (Faix and Beinhoff, 1988) and the 1147 cm^{-1} band is assigned to C-O-C stretching, since it is an acceptor of

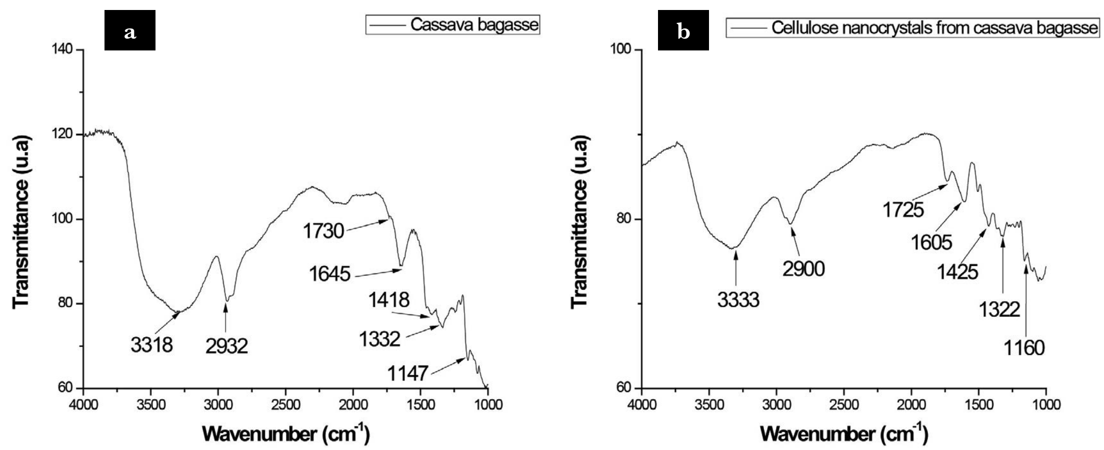

Figure 1. Interferograms (FTIR) of: a) cassava bagasse (*M. esculenta*) and b) cellulose nanocrystals.

protons capable of forming hydrogen bonds with proton donors, such as OH in cellulose nanocrystals and cellulose nanofibers (Kakade *et al.*, 2007). Figure 1b shows the peaks of absorption close to 1605 cm^{-1} associated with the C-C aromatic bond in the symmetric stretching to the vibration plane of the aromatic ring present in lignin (Garside and Wyeth, 2003; Wang *et al.*, 2009). Villar (2010), Bourtoom and Chinnan (2008), and Kim and Lee (2002) report that the 1425 and 1322 cm^{-1} peaks (of nanocrystals) correspond to bending vibrations of C-H, and the 1160 cm^{-1} band is attributed to the asymmetric stretching of cellulose C-O-C (Grande, 2014), specifically cellulose nanocrystals.

Physical characterization via X-ray diffraction (XRD)

In the X-ray diffractogram (XRD) of the cassava bagasse (Figure 2a) the following peaks are identified: 15.1, 17.1, 18.1 and 23.1°, which represent the typical diffraction pattern of type A crystals.

This type of crystallinity is more susceptible to enzymatic hydrolysis and is found in the starches of cereals and some roots, as well as in tubers such as cassava, potato and jicama

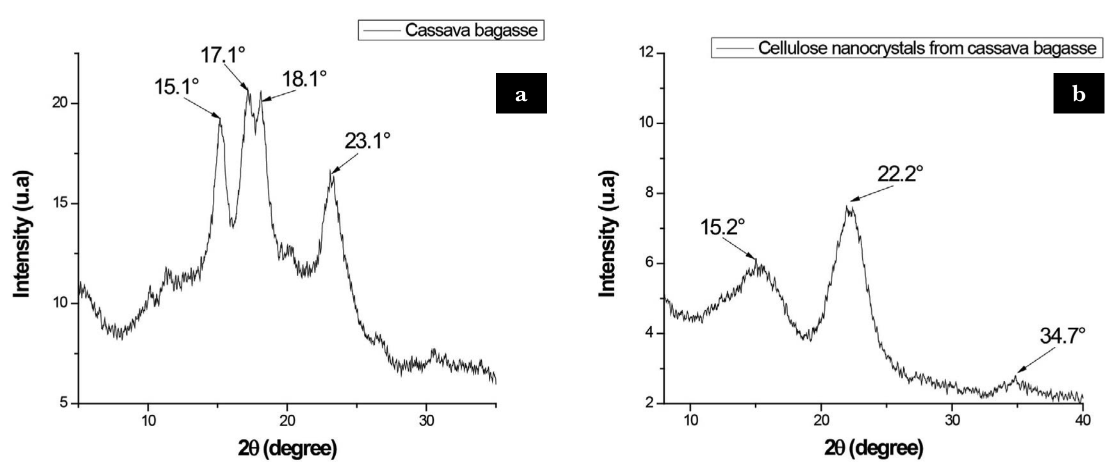

Figure 2. Diffractograms (XRD) of: a) cassava bagasse (*M. esculenta*) and b) cellulose nanocrystals.

(Flores, 2004; Atichokudom *et al.*, 2001). The percentage of crystallinity obtained from Eq. 1 was 37.1%, which is higher to that reported in cassava bagasse obtained from the Purbalingga, industry in Indonesia (14.52%) (Wicaksono *et al.*, 2013), savannah cassava (23%) and emerald (26%) starch (Vargas, 2015), and lower to that from sugarcane bagasse (43.6%). In the 15.2, 22.2 and 34.7° peaks present in the cassava cellulose nanocrystals (*M. esculenta*) (Figure 2b), an increase in intensity is observed, indicating greater crystallinity (47.9%), due to the elimination of amorphous material (hemicellulose and lignin). The 22 and 34° peaks belong to the reflection of the lattice on different planes (Sassi and Chanzy, 1995). Sugiyama *et al.* (1991), mention that values similar to 2=14.9°, 16.7°, 20.6°, 22.8° and 34° exhibit the type I crystalline structure which is found in nature and commonly known as native cellulose (I′), which is predominant in plants.

Morphological characterization and elemental analysis (SEM-EDX)

Figure 3a presents SEM micrographs obtained, where average diameters of 2 μm can be observed in cassava bagasse (*M. esculenta*). Moron *et al.* (2017) report diameters that range from 0.5 to 2.52 μm for this same material, while Versino *et al.* (2015) found

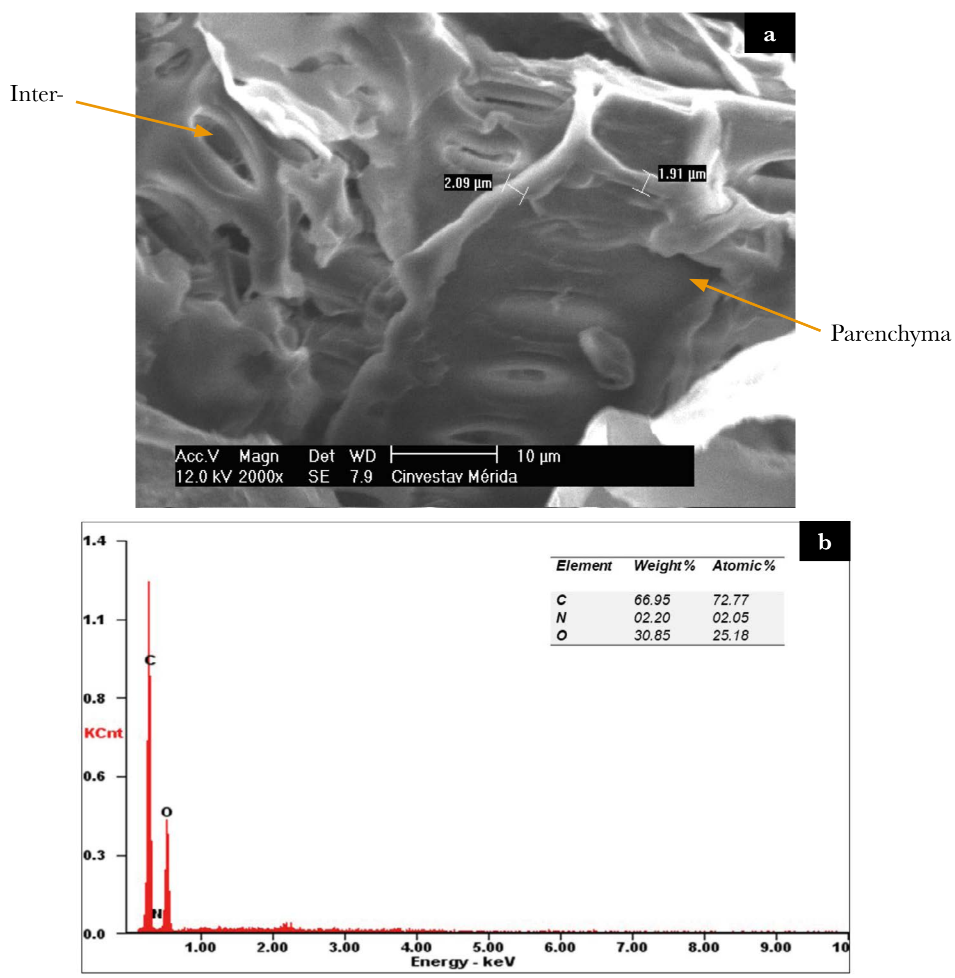

Figure 3. a) SEM micrographs of cassava bagasse and b) elemental analysis.

that the peels had bigger particles (mainly 300 μm) compared to the bagasse (particles smaller than 53 μm) while conducting studies with cassava (*M. esculenta*) bagasse and peel. The variations found can be attributed to the grinding and sifting processes prior to the micrograph sampling. The images clearly show the parenchyma, as well as the detachment of some of the fibers as a result of the grinding.

The SEM-EDS elemental analysis carried out on cassava bagasse (Figure 3b) demonstrates as principal components: carbon (C), oxygen (O) and traces of nitrogen (N). Cours *et al.* (1961) report the percentage in detail (in dry cassava) of N, K, P, and Ca in cassava leaf blades, petioles and branches, wood, and phelloderm of stems. Their results indicate that the percentages of nitrogen range from 3.84 to 0.76%. Lozano *et al.* (1981) report that the leaf blades have a higher content of N and P, the petioles of K and Ca, and add that the nutrient content in the same tissue changes as the plant ages, since in the case of cassava the contents of N, P and K decrease while the contents of Ca and Mg increase during the growth cycle.

Characterization of the diameter and amplitude of cellulose nanocrystals with an atomic force microscope

The AFM micrograph carried out on CNC (Figure 4), obtained via hydrolysis with HCI, shows rod-shaped nanocrystals with a helical appearance and no residual charge, having

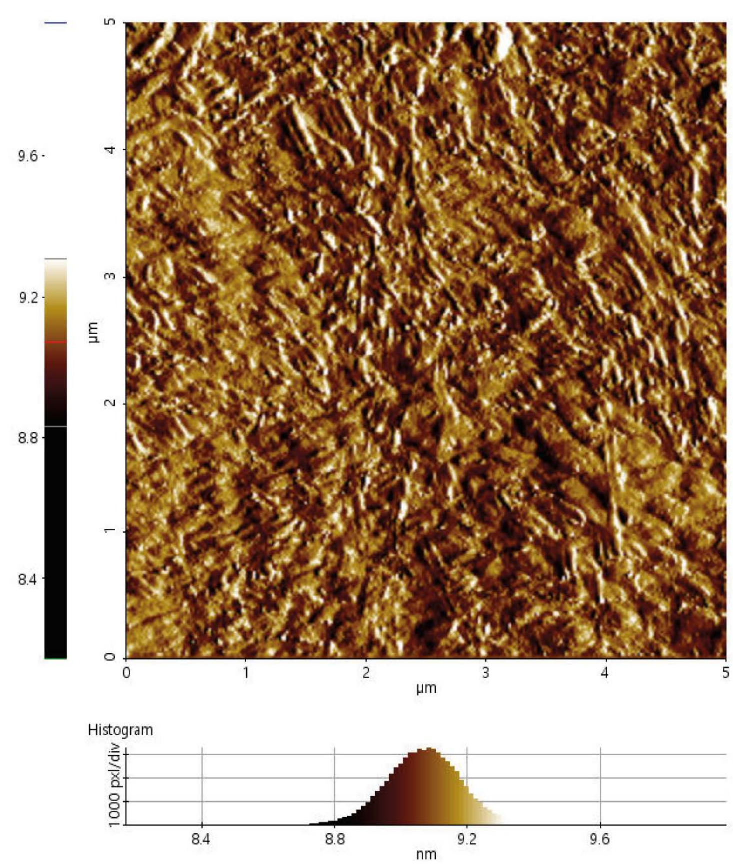

Figure 4. AFM micrograph of the cassava bagasse CNC.

a diameter of 8.7 and 9.3 nm. Teixeira *et al.* (2009) report values of 25±7 nm in cassava bagasse cellulose nanofibers, obtained through H_2SO_4, hydrolysis, while Wicaksono *et al.* (2013) report smaller diameters (5-8 nm), with the combination of chemical treatments (alkaline solutions to hydrolyze pectin and hemicellulose), as well as mechanical.

It is important to point out that the characteristics of cellulose CNC can be affected primarily by the raw material employed, the methodology (parameters like time, temperature, and reagents), types of treatments (chemical and mechanical) and the equipment used during the process. The cellulose CNC prepared with HCI have a limited capacity to be dispersed and their aqueous suspensions tend to flocculate (Araki *et al.*, 1998), while those prepared with H_2SO_4 lead to more stable aqueous whisker suspensions, presenting a higher negative charge on their surface, due to the formation of sulfate groups during the acid treatment in comparison to those prepared with HCl (Gardner *et al.*, 2008; Peng *et al.*, 2011).

CONCLUSIONS

Characteristic functional groups were identified, as well as the bonds present in cassava bagasse and in the cellulose nanocrystals obtained from the anhydrous glucose units of amylose and amylopectin, lignin, hemicellulose, holocellulose, and water; the presence of cellulose type I was corroborated, which is found in nature and commonly known as native cellulose ($I\beta$). The crystallinity percentages of the nanocrystals indicate that the amount of lignin and hemicellulose present decreased; however, they also suggest that the amorphous cellulose dominions remain to a greater extent. It was possible to obtain cellulose nanocrystals from cassava bagasse (*M. esculenta*), with rod shape, helical appearance and no residual charge. However, it would be interesting to attempt their formation with different methodologies, managing to increase the percentage of crystallinity and with this, its field of application.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

The orchids of Megamexico and their interactions with pollinators

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ABSTRACT

Objective: To describe the pollination syndromes of the orchids of Megamexico and the importance of the interactions between the orchids and their respective pollinators for the conservation of both groups.

Design/Methodology/Approach: An exhaustive search was carried out on the pollinators of each of the orchids that grow in Megamexico. With the information sources available, a data matrix was prepared that includes orchids and all their pollinators. Subsequently, it was quantified which group of pollinators the orchids interact with the most. Finally, it is described what physiological adaptations and morphologies orchids have developed to attract specific pollinators.

Results: Orchids from Megamexico maintain close relationships with specific pollinators. Said interaction is mediated by the shape, size, production of aromas, nectar, and the color of the flower. Thus, four large groups of pollinators are those that interact with the orchids of Megamexico, with the Hymenoptera being the group of pollinators that pollinates the most orchid species in Megamexico and birds to a lesser extent.

Study Limitations/Implications: This study describes the importance of pollinators and their interactions with orchids for orchid prevalence.

Findings/Conclusions: It is of vital importance to include orchid pollinators in conservation programs to ensure that interactions between orchids and pollinators continue to be effective and thus guarantee the permanence of the two groups.

Keywords: Conservation, ecological interactions, Megamexico, pollination syndrome.

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INTRODUCTION

Megamexico is a biogeographic region that stands out in the world for having an important diversity and endemism of plants (Rzedowski, 1991). Among this diversity, the Orchidaceae family stands out, which turns out to be an important component of the vegetation of this biogeographic region (Ames and Correl, 1985; Hágsater *et al.*, 2005; Gutiérrez-Rodríguez *et al.*, 2022). Orchidaceae is the most diverse family of angiosperms

Image by David at Pixabay

(Christenhusz and Byng, 2016). It is made up of 30,000 species grouped into 736-880 genera (Chase *et al.*, 2015). Due to its way of life, it contains plants that are epiphytes (which grow on trees without causing them any damage), lithophytes (grow on rocks), geophytes (grow on the ground) and saprophytes (they are terrestrial, but they need fungi to survive).

THE DIVERSITY OF ORCHIDS IN MEGAMEXICO

In Megaméxico the presence of 1,732 species is reported, which in turn belong to 189 genera, 36 subtribes and 17 tribes (Gutiérrez-Rodríguez *et al.*, 2022). This great diversity has been possible thanks to climatic heterogeneity, topographic complexity, geological history, and the vast diversity of vegetation types in Megaméxico, which are some of the conditions that have favored the diversification and prevalence of many orchid species (Gutiérrez -Rodriguez *et al.*, 2022).

The diversity of existing species in Orchidaceae is a product of their ability to exploit a wide range of ecological niches (Ricklefs and Renner, 1994), their habitat specialization (Gravendeel *et al.*, 2004) and the ecological interactions that occur in this group. Several studies have linked orchid diversity to specialized interactions with mycorrhizal fungi and to a greater extent with specific pollinators (van der Pijl and Dodson, 1966; Dressler, 1981; Tremblay, 1992; Cozzolino and Widmer, 2005 Jersáková *et al.*, 2006; Borba *et al.*, 2011; Zhang *et al.*, 2017).

IMPORTANCE OF INTERACTION WITH POLLINATORS

Without a doubt, the flower is the most attractive part of orchids, which is why, together with its structures, they have been the subject of numerous studies (*e.g.* Dressler, 1961; van Der Pijl and Dodson, 1966; Castro and Singer, 2019). Notably, Charles Darwin (1885) was one of the first to examine orchid flowers and their pollination strategies. Orchids depend on their floral morphology to be pollinated (Schiestl and Schlüter, 2009). For this purpose, they have developed flowers with very particular shapes, structures, colors, and fragrances that are important in the life cycle of orchids to attract pollinators and thus achieve pollination (Hágsater *et al.*, 2005; Castro and Singer, 2019).

Orchids are one of the most amazing and intriguing examples of pollinator interactions (Tremblay, 1992) and are among the most specialized plants with respect to their pollination (Schiestl and Schlüter, 2009; Phillips *et al.*, 2009, 2011; Castro and Singer, 2019; Lipińska *et al.*, 2022). It is estimated that about 60% of orchids interact with a single species of pollinator (Tremblay, 1992). This suggests that there is an important adaptation process in this family towards pollinators (Cozzolino and Widmer, 2005). The fascinating interaction between plants and pollinators is driven by different interests. Orchids need reliable pollen dispersal and pollination at minimal costs; while pollinators seek floral rewards that can be harvested as quickly and efficiently as possible (van der Kooi *et al.*, 2021).

Orchids display various adaptations that restrict access to floral rewards in many ways, from morphological to mechanical, including chemical barriers. Furthermore, pollinators were not left behind and developed strategies that allowed them to maintain the exploitation of floral resources to their benefit (van der Kooi *et al.*, 2021). Among these strategies there are morphological, behavioral and/or physiological modifications (Danfort *et al.*, 2019). Just to mention a few of the adaptations is body size that determines whether or not a pollinator can enter the flower and access the rewards. While physiological adaptations allow pollinators to exploit rewards efficiently and without having negative effects on reward toxicity (Danfort *et al.*, 2019). The success of pollination depends on how the pollinator interacts with the sexual organs of the flower, something that also depends on its morphological and temporal adjustment (Simon Porcar *et al.*, 2018).

ORCHID POLLINATION STRATEGIES

Pollinators have played an important role in the evolution of the Orchidaceae, and this is evidenced by the complex pollination mechanisms that are unique to orchids (van der Pijl and Dodson, 1966; Tremblay, 1992). This relationship between orchids and pollinators has given rise to a great diversity of floral morphologies that have been linked to specialization in pollination (van der Pijl and Dodson, 1966; Dressler, 1981; Tremblay, 1992; Jersáková *et al.*, 2006).

Orchids have developed strategies to ensure pollination and thus have effective propagation (van der Pijl and Dodson, 1966b). As in other plant families, orchids produce flower oils, nectar, and perfumes in order to attract pollinators (Ackerman, 1986; Dressler, 1982). In orchids, the production of oils and resins only occurs in five subtribes: Bifrenariinae, Catasetinae, Cyrtopodiinae, Maxillariinae and Oncidiinae. It should be noted that all are exclusive to the Neotropics. Similarly, the number of flowers, the size of the inflorescence and the density of the flowers play an important role in the pollination process (Willmer, 2011). Among the pollinators of orchids are: Coleoptera (beetles), Diptera (flies and mosquitoes), Hymenoptera (bees, bumblebees, and wasps), Lepidoptera (butterflies and moths) and some birds (hummingbirds). Likewise, some orchids present self-pollination (van der Pijl and Dodson, 1966; Williams, 1982).

Various studies indicate that more than 30% of orchids are pollinated by deception systems (van der Pijl and Dodson, 1966; Ackerman, 1986b; Tremblay *et al.*, 2005; Jersáková *et al.*, 2006). Orchids that use deception to attract pollinators employ different mechanisms ranging from feeding deception to floral and sexual mimicry (several times leading to pseudocopulation) (Jersáková *et al.*, 2006). This deception consists of imitating odors and visual stimuli to deceive pollinators that are looking for food or reproduction (Cozzolino and Widmer, 2005). Thus, non-reward orchid flowers have a complex morphology, in many cases having the appearance of the female pollinator. In this case it is known as sexual deception syndrome (van der Pijl and Dodson, 1966; Dressler, 1993; Ayasse, 2006). On the other hand, when there are structures in the flower that resemble food and that deceive the pollinator, it is known as food deception syndrome (Ackerman, 1986a, b).

POLLINATION SYNDROMES IN ORCHIDS

In Orchidaceae, it has been documented that each subtribe or even each genus have specific pollinators according to the characteristics that the flowers present (van der Pijl and Dodson, 1966; Tremblay, 1992; Bogarin *et al.*, 2018). For example, it has been reported that flies are the pollinators of the Pleurothallidinae (Albores-Ortiz and Sosa, 2016; Bogarin *et al*., 2018); Euglossine bees are pollinators of Catasetinae, Stanhopeinae and some representatives of Beltiinae, Lycastinae, Oncidiinae and Zygopetalinae; all from the Neotropics (Dressler, 1968; Parra *et al*., 2016). On the other hand, some members of Habenariinae are pollinated by Lepidoptera and Orchidinae by Hymenoptera (Inda *et al*., 2012), just to mention a few. Furthermore, orchids may share pollinators with other orchids or even with plants from other families (Ackerman, 1983).

Pollination efficiency is tied to how the pollinator interacts with the sexual organs of the flower, which is determined by its morphological and temporal adjustment (Simón-Porcar *et al*., 2018). Pollination mechanisms in orchids have been extensively studied and have been grouped according to taxonomic groups of pollinators and flower morphology in the so-called "orchid pollination syndromes" (van der Pijl and Dodson, 1966; Tremblay, 1992). These syndromes clearly show the adaptations between the flower and the animals that pollinate them (van der Pijl and Dodson, 1966) (Table 1). The main characteristics of each of the groups of pollinators are described below.

Table 1. Comparison of pollination syndromes of orchids from Megaméxico.

Syndrome	Pollinator	Morphological adaptations of orchids	Flower coloring	Physiological adaptations	Notes	Examples
Melittophilous	Bees	Zygomophic flowers with complex nectar guides and platform-shaped lip for landing	Violet, blue, green, yellow, and white	Flowers that open during the day (may or may not close at night). Pleasant and fresh aromas	Some instead of nectar produce volatile essences	*Bletia, Laelia, Sobralia, Stanhopea, Vainilla*
Phalenophilous	Moths	Pollinia attach themselves to the feet of pollinators. No nectar guides. Flower does not erect, usually pendulous	White, cream, or greenish	Sweet scent and flowers open at night	They have short nectar tubes	*Epidendrum parkinsonianum, Coenoemersa, Habenaria, Platanthera*
Phsychophilous	Butterflies	Contain nectar in long narrow tubes, erect flower position, Zygomorphia not necessary	Bright colors, red, orange, yellow, pink, purple	The flowers open during the day. Pleasant and fresh aromas. Abundant nectar, usually in deep containers	Pollen sticks to the legs of pollinators	*Bletia, Epidendrum, Funkiella, Sacoila*
Ornithophilous	Birds	Nectar deep and occasionally protected. Pollen can be brown or dark colors	Predominant reddish colors. Red, orange, pink	No aroma or very faint aromas; hanging flowers	Pollen adheres to the beak	*Alamania, Arpophyllum, Comparettia falcata, Dichromanthus, Stenorrhynchos*
Miophilous/ sapromiophilous	Flies	Small flowers with transparent structures and may have hairs. Sometimes with mobile organs to the wind	dark purple, brown, and yellow colors with spots and lines	Strong aromas of decomposing matter	No nectar guides	*Bulbophyllum, Lepanthes, Pleurothallis, Stelis*
Cantharophilous	Beetles	Large flowers, sac-shaped lip	Mostly Yellow, green, purple	Nectar-producing	They usually eat part of the flower	*Cypripedium*

Hymenoptera

Bees, bumblebees, and wasps (Hymenoptera) are undoubtedly the taxonomic group that pollinates the most species of orchids, mainly members of the Apidae, Colletidae, Halictidae and Megachilidae tribes; and it is the only group that interacts with members of all five Orchidaceae subfamilies. Orchids took advantage of the great diversity of Hymenoptera and their cognitive abilities to diversify in the same way (Mondragón-Palomino and Theißen, 2009; Ramírez *et al.*, 2010). The orchid-hymenopteran interaction is one of the most fascinating in nature (Figure 1A). This great variety of mechanisms of attraction to pollinators can be through the generation of a) aromatic oils, b) nectar, c) nectar deception or d) sexual deception.

Smells play an important role in attracting some species of pollinators. One of the most studied cases is that of Euglossine bees (Apidae: Euglossini), which are known to actively collect floral perfumes that they probably use during courtship (Ramírez *et al.*, 2002; Eltz *et al.*, 2005). Within Orchidaceae, this pollination mechanism is restricted to the Epidendroideae subfamily, within the Catasetinae, Stanhopeinae subtribes and part of the Zygopetaliinae and Oncidiinae (Castro and Singer, 2019).

The Euglossini are exclusive to the Neotropics, they are distributed from Mexico to northern Argentina (Parra *et al.*, 2016). These bees learn and remember the chemicals they collect, their innate odor preferences and memory seem to prevent over-collection (Eltz *et al.*, 2006). It has been highlighted that the olfactory preferences of male euglossine bee species are a strong selection pressure towards orchid species (Roubik and Hanson, 2004). For this reason, orchids emit these substances to attract euglossine bees through a great diversity of aromas that vary intra- and interspecifically (Hetherington-Rauth and Ramírez, 2016).

Orchids pollinated by euglossine bees develop osmophores on the labellum (Franken *et al.*, 2016). High humidity and temperature levels can increase the metabolism of the scent glands (osmophores) of orchids, which translates into an accentuation of aromas (Téllez-Velasco, 2013). The orchids that emit these aromas grow mainly in tropical regions and their emission is generally higher in the morning, where the activity of euglossin bees is higher (Rodríguez-Flores *et al.*, 1995).

When male euglossin bees collect volatile compounds in the pollination process they are aided almost entirely by the intricate floral morphology of the orchid, which normally places the pollinator in the proper position. This position ensures precise placement of the orchid pollinarium in a specific area of the bee body, which in turn ensures successful deposition of the pollinia on the stigma later (Dressler, 1982).

On the other hand, some orchid species provide nectar as a reward to attract bees. Generally, in these orchids, the lip wraps around the column, forming a wide funnel-shaped tunnel into which the pollinator has to penetrate to carry out pollination. This type of flower is sometimes referred to as the throat type and is present in several groups of orchids not necessarily related, such as the genera *Bletia*, *Sobralia*, *Laelia* or *Cattleya*.

To attract bees, orchids also employ feeding deception, where they exploit the innate foraging behavior of pollinators. In this type of pollination, orchids signal that they are rewarding plants, such as the shape of the inflorescence, the color of the flower, the aroma, the nectar guides, and the spurs, this is usually especially effective for the bumblebees of the genus *Bombus* (Kunze and Gumbert, 2001). They also tend to flower gregariously in early spring, exhibit flower color polymorphism, and exploit newly emerged bees and bumblebees after hibernation (Heinrich, 1975). This strategy is particularly successful, since it is estimated that one third of orchids present this feeding deception mechanism (close to 10,000 species) (Ackerman, 1986a). Several experimental studies have shown that feeding cheating promotes cross-pollination, thus favoring genetic diversity, however, we still do not understand how it became such a successful strategy (Jersáková *et al.*, 2006).

Finally, orchids can attract pollinators in a spectacular form of floral mimicry: sexual deception. Hitherto known exclusively in orchids, the flowers mimic the mating signals (physical or chemical) of certain insect species and are pollinated by sexually excited males, who mistake the flower for a female and pollinate it during "pseudocopulation" (Jersáková *et al.*, 2006). Orchid species that perform sexual deception may have widely open flowers that expose the lip, which mimics or at least part of the female insects. Interestingly, many sexually deceptive orchids have flowers with green, red or white colorations (Gaskett, 2011).

Within Orchidaceae, this pollination strategy is exclusive to the Orchidoideae and Epidendroideae subfamilies (Castro and Singer, 2020). Examples of this fascinating interaction of orchids with hymenoptera are orchids of the genera *Lepanthes* (Blanco and Barbosa 2005), *Trigoninium*, *Mormolyca* (Singer *et al.*, 2004), *Telipogon*, *Tolumnia* and *Trichoceros* are pollinated by the melipona bee *Plebeia droryana* (Apidae: Meliponini) (Singer, 2002; Chase, 2009; Martel *et al.*, 2016).

Lepidoptera

Pollination syndromes that are carried out by Lepidoptera are divided into two groups: sphingophilic when pollinated by moths and psychophilous when pollinated by butterflies (van Der Pijl & Dodson 1966). Lepidoptera pollinate about 10% of orchids, of which 85% are nectar-producing species (Figure 1D). Thus, the orchid flowers that are characteristic of this pollination syndrome are those that produce nectar; on which these pollinators feed. The flowers are adapted with a tubular-shaped nectary where pollinators enter their mouthparts called a proboscis for nectar absorption. Orchids that present this pollination syndrome are more common in tropical areas.

The sphingophilic flowers usually present white, beige or very light greenish colors. Likewise, the flowers usually open at night and have odors that are attractive to moths. Examples of this syndrome are the species of *Platanthera*, *Habenaria* and some *Epidendrum*, to mention a few. For their part, psychophilic flowers present bright colors such as orange, red, pink, purple, etc. They contrast with the sphingophiles by opening their flowers during the day and producing fresh aromas that attract butterflies. Some species of *Epidendrum*, *Sacoila*, *Funkiella* and some *Bletia* are examples of this pollination syndrome.

Thus, the orchids that are pollinated by Lepidoptera all have a structure that resembles a small tube, which is located at the base of the lip, whether it is spur-shaped as in *Habenaria* or a narrow tunnel product of the fusion of the column with the base of the lip as in many species of *Epidendrum* (Hágsater *et al.*, 2005). These small tubes are adapted to be penetrated

Figure 1. Representation of pollination groups in orchids. A) Pollination by Hymenoptera (*Stanhopea tigrina* and *Euglossa viridissima*), B) Birds (*Comparettia falcata* with *Chlorostilbon maugaeus*), C) Diptera (*Lepanthes glicensteinii* and *Bradysia floribunda*) and D) Lepidoptera (*Epidendrum veroscriptum* and butterfly of the family Pieridae).

only by the pollinator's proboscis or beak. The flowers that present these structures are called "key-lock" flowers (Dressler, 1981). In several species, this tunnel enters the ovary or the pedicel and at the bottom there is a larger cavity that contains the nectar. The length of the tunnel has adapted to the size of the pollinator's proboscis. *Brassavola* and *Rhyncholaelia* are obvious examples of this situation.

Diptera

After Hymenoptera, the Diptera are the second most important group of orchid pollinators. Pollination carried out by flies and mosquitoes is called myophily (van der Pijl and Dodson, 1966; Christensen, 1994). Myophily is considered one of the most promiscuous and widespread pollination syndromes in angiosperms (van der Pijl and Dodson, 1966). Diptera participate in the pollination of the richest tribes in species of the family such as Dendrobiinae, Malaxidinae and Pleurothallidinae (Ackerman *et al.*, 2022).

The flowers that present this pollination syndrome are very small and with different colors ranging from purple-pink and yellow with spots and lines. Furthermore, they do not produce nectar to attract pollinators (Borba *et al.*, 2011). In this case, the flowers produce fetid odors, which in many cases are not perceptible to humans, but are easy for flies to smell since they resemble rotten meat, decomposing fruits, etc. These orchids imitate being the food of the diptera that pollinate them. In many cases, the odor they emit attracts very specific pollinators (Christensen, 1994). An example of this syndrome is the species of *Stelis* where many of the species of the genus have dark colors in their flowers and frequently develop mobile structures and fetid odors (Figure 1C).

Lepanthes species are known to use sexual deception and are pollinated by fungus gnats of the family Sciaridae. The male mosquitoes, by confusing the flowers with the female mosquitoes, try to copulate with the flowers where, in addition, their genitals fit exactly in the appendages of the lip as they would with the female sexual organs of the female mosquito. Besides being amazing, this case demonstrates the incredible morphological adaptation of orchids to being pollinated by specific pollinators. All Pleurothallidinae and Cypripedioideae are examples of this type of pollination along with species belonging to the genera *Epipactis*, *Habenaria*, *Liparis*, *Malaxis*, *Corallorhiza*, to name a few.

Birds

Orchid pollination by birds has only been reported in tropical regions (Ackerman *et al.*, 2022). In Megaméxico, hummingbirds (Trochilidae) are the only birds that pollinate orchids where members of the two largest subfamilies, Epidendroideae and Orchidoideae, pollinate (Figure 1B). Few orchid species (around 3% of species) have interactions with these fast-moving birds. However, most of the orchids that are pollinated by hummingbirds have some interesting and unique characteristics that we will mention below: These orchids are usually from high and/or cold areas, since a large part of the pollinating insects do not reach these areas because that do not have the ability to thermoregulate, this is an opportunity that hummingbirds that, having said ability, can take advantage of. Hummingbird-pollinated orchids tend to have bright colors with predominantly reddish tones, such as bright red, pink, purple, orange, yellow, or magenta. Most insects cannot perceive the red of the color spectrum and hummingbirds, having the ability to see those colors, can take advantage of this (Siegel, 2011).

Ornithophilous orchids usually have a callus that partially closes the floral tube at the level of the anther and the stigma (van Der Pijl and Dodson, 1966). The pollen sacs of most orchids are yellow, but this yellow would produce a high visual contrast with the color of the bill and hummingbirds would be stimulated to clean their bills, implying crossbreeding failure in orchids (Lipińska *et al.*, 2022). That is why, to avoid detection, a large part of the orchids pollinated by hummingbirds evolved to have a dark pollinarium (Dressler, 1971). Some examples of hummingbird-pollinated genera that have some of the characteristics mentioned above are *Elleanthus* and *Comparettia*. It is important to note that the curvature of some flowers coincides with the curvature of the beaks, which makes pollination more efficient (Castro and Singer, 2019).

Other Animal Interactions

Orchids not only interact with pollinating animals, but also with species with very particular functions. For example, some orchids of the genus *Myrmecophila* have hollow pseudobulbs that serve as homes for ants (Thien and Rico-Gray, 2004). These orchids in their mutualistic relationship provide shelter and food for the ants and these defend the plants from being defoliated by herbivores and fertilize the soil (Hágsater *et al.*, 2005).

These pseudobulbs have been reported to harbor at least nine species of ants, each species living in a different pseudobulb (Thien and Rico-Gray, 2004). This relationship can become so relevant that some orchids only grow directly on anthills, forming so-called ant gardens (Kleinfeldt, 1978). For example, *Epidendrum flexuosum* and *Coryanthes picturata*, the interaction with ants is essential and they are obligate myrmecophilous orchids, since for both species the anthills are a favorable environment for the germination and growth of these species while the roots of the plants give structure to the anthills and protect it (Morales-Linares *et al.*, 2018).

THE POLLINATORS OF THE ORCHIDS OF MEGAMEXICO

In Megaméxico, bees are the main pollinators of orchids; pollinate 827 species of orchids, it should be noted that it interacts with species from the four Orchidaceae subfamilies present (Figure 2). The second most important group are flies pollinating 473 orchid species, followed by butterflies and to a lesser extent by hummingbirds and beetles (Figure 3). As for orchids, the Epidendroideae subfamily is the richest in species in the family and they present a wide range of shapes and colors in their flowers. Within this subfamily, each subtribe has particular traits and morphological adaptations of the flowers that allow them to be pollinated by certain groups of pollinators. For this reason, it is the group that interacts with all the groups of pollinators present in our study region (Figure 2). In Orchidoideae it interacts with all pollinators except wasps and beetles. In the case of Cypripedioideae it is pollinated by Diptera and beetles. Finally, the Vanilloideae only interact with bees

CONSERVATION IMPLICATIONS

Megamexico orchids and their respective pollinators coexist due to the interactions that occur between them. However, these interactions are threatened since the habitats of

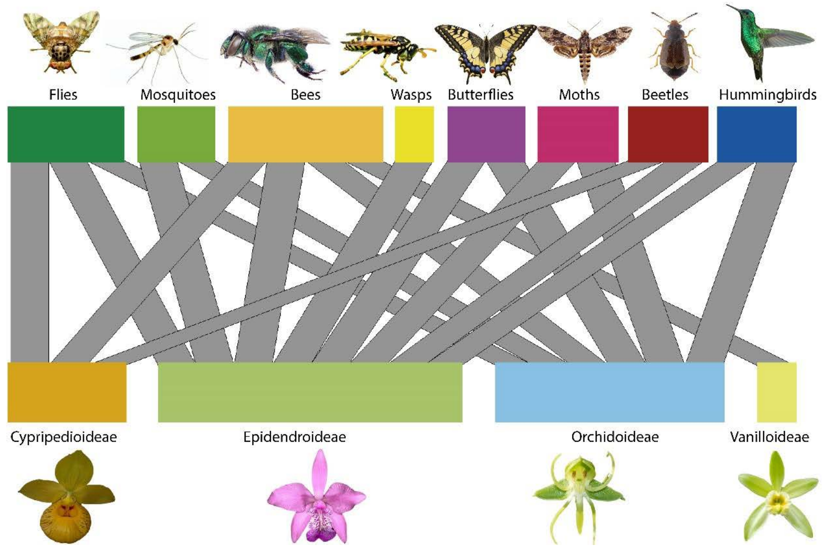

Figure 2. Network of interactions of orchids and pollinators of Megamexico. Lines represent interactions between pollinator groups and Orchidaceae subfamilies.

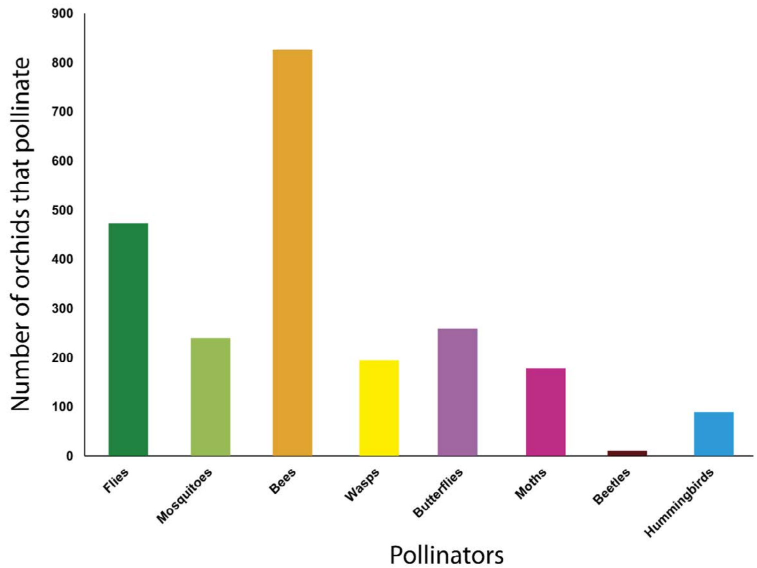

Figure 3. Representation of the number of orchids pollinated by each of the groups of pollinators.

the orchids and therefore of the pollinators are in danger; the causes and consequences of the loss of orchid habitats are listed in Gutiérrez-Rodríguez (2022). The disappearance of orchids or their pollinators will inevitably lead to the extinction of one, the other, or both. Both orchids and pollinators are affected to varying degrees by environmental fluctuations and disturbances in ecosystems. Therefore, habitat conservation is a critical factor that affects their permanence and, in that sense, their survival. We believe that an ecological study of orchid-pollinator interaction networks is imperative to understand how these interactions are structured, which are vital for the life cycles of both groups of organisms. The study of interactions between orchids and pollinators provides a fundamental basis for conservation proposals considering the habitat requirements not only of orchids but also of the organisms with which they interact.

We also believe that it is extremely important to call for more research on orchid pollination, since many groups have not been studied and little or nothing is known about their pollinators. In this sense, we encourage researchers and people in general to contribute to this gap that is little explored for many groups and we invite them to make more field observations on orchid pollinators and report them through the use of digital platforms such as the website of Naturalista (https://www.naturalista.mx/). Finally, we believe that it is very important to include orchid pollinators in conservation programs to ensure that interactions between orchids and pollinators continue to be effective and thus guarantee the permanence of the two groups.

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