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Optimization of the composting process of sugarcane filter-pressed mud in the Santa Rosalia sugar mill, Tabasco, Mexico

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ABSTRACT

Objective: To optimize the composting process of sugarcane filter-pressed mud, straw, and ash as an alternative to reduce the environmental contamination of sugarcane cultivation in the Santa Rosalía sugar mill (Ingenio Santa Rosalía) of Chontalpa, Tabasco, Mexico.

Design/Methodology/Approach: A completely randomized experimental design in a factorial arrangement was used, with compost mixtures and the aeration times as study factors. Ten treatments were generated with six repetitions each. The study variables were pH, organic matter (%), and nitrate $\left(NO_3^-\right)$ and ammonium $\left(NH_4^+\right)$ content.

Results: The compost mixture factor had significant effects on compost quality parameters. Aeration times did not affect the quality of the different compost mixtures. Chemical characteristics of pH and organic matter did not present differences between the treatments.

Study Limitations/Implications: The use of industrial sugarcane residues generates quality organic fertilizers through the composting process; therefore, it is necessary to test more residues, such as molasses and vinasses.

Findings/Conclusions: The 100% sugarcane filter-pressed mud and the 100% sugarcane filter-pressed mud+0.5% N treatments presented the highest amount of NO_3^- and NH_4^+ and were therefore considered the best treatments. All treatments were classified as mature composts, rich in organic matter, with alkaline pH, and made of steady materials.

Keywords: Compost, waste, sugarcane filter-pressed mud, quality, sugarcane.

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INTRODUCTION

Sugarcane (*Saccharum* spp.) is an important crop in Mexico, due to the monetary incomes and jobs it generates (Palma-López *et al.*, 2016). From 2007 up to date, Ingenio Santa Rosalia (ISR), located in the region of Chontalpa, Tabasco, has increased its cultivated area from 9,038 to 13,012 ha. Its cane yield has likewise increased from 41.6 to 67 t ha^{-1}, although they still are lower than the national average (68.7 t ha^{-1}) (CONADESUCA, 2020). In order to increase productivity, sugarcane crops in Mexico and in the state of Tabasco are generally subjected to burn, mechanization, and an excessive use of fertilizers. These activities have several adverse effects, including:

reduction of soil organic matter, increase of erosion, and decrease in the density of microbial populations that are beneficial for crops (Palma-López *et al*., 2016). The use of compost or organic fertilizers, biofertilizers, green manures, and covers crops is an alternative for soil restoration (Pérez *et al*., 2011). Sugarcane generates excess organic matter such as vinasse, bagasse, and sugarcane filter-pressed mud. Although sugarcane filter-pressed mud is used in the ISR, it has not been possible to optimize its composting and, consequently, to reduce production and application costs (Salgado *et al*., 2013). The contribution of these modifications can help preserve and promote the structure of the soil, because organic matter favors aggregation in clay soils through physical and chemical mechanisms. This type of soils includes vertisols, fluvisols, and gleysols, which have low aggregation and poor drainage problems (Sánchez Hernández *et al*., 2006; Palma-López *et al*., 2016; Salgado *et al*., 2013) that are typical of ISR area.

MATERIAL AND METHODS

Origin of compost

The collection of materials for the 2019-2020 composting was performed on May 2021 in the area of influence of ISR in la Chontalpa, Cárdenas Tabasco, at the end of the harvest season. The newly generated sugarcane filter-pressed mud was left outdoors for 30 days for cooling. Sugarcane straw were collected 4 days after the harvest of a commercial plot (4 days of sun exposure) and fractionated with a forage mill. Ash was obtained from the ISR boiler and FYPA® urea was applied as a nitrogen source (46% N).

Experimental site

The composting process was established indoors at the facilities of the Universidad Popular de la Chontalpa, located at km 2 of the Cárdenas-Huimanguillo road, in the municipality of Cárdenas, Tabasco, Mexico.

Treatments and experimental design

The treatments were developed using a completely randomized design with 5×2 factorial arrangement (5 compost mixtures and 2 aeration times: 7 and 14 days), which generated 10 treatments (Table 1) that were distributed randomly in the experimental site with six replicates (Figure 1). Sixty 38-kg experimental units were generated. A 20-liter bucket was used to mix the compounds of the different experimental units and scales were used for the measurement reference. For the application of the sugarcane filter-pressed mud only the scales were used.

Handling of the composting process

The treatments were established in a site with a concrete floor and ceiling on a 2×2 m plastic film (Table 1). The materials were mixed for homogenization over the film (Figure 2a). Subsequently, they were wrapped with the same nylon to protect them and prevent leaching (Figure 2b); the treatments were subject to an aeration process every 7 or 14 days.

Table 1. Study treatments with different mixtures and aeration times.

Treatments	Compost mixtures	Aeration time (days)
1	Sugarcane filter-pressed mud 100%	Seven days*
2	Sugarcane filter-pressed mud 100% + 0.5% N*	Seven days
3	Sugarcane filter-pressed mud 75% + bagasse 25%	Seven days
4	Sugarcane filter-pressed mud70% + 20% straw + 10% ash	Seven days
5	Sugarcane filter-pressed mud 60% + 30% straw + 10% ash	Seven days
6	Sugarcane filter-pressed mud100%	Fourteen days
7	Sugarcane filter-pressed mud 100% + 0.5% N*	Fourteen days *
8	Sugarcane filter-pressed mud 75% + bagasse 25%	Fourteen days
9	Sugarcane filter-pressed mud 70% + 20% straw + 10% ash	Fourteen days
10	Sugarcane filter-pressed mud 60% + 30% straw + 10% ash	Fourteen days

*Applied as FYPA urea, with a 46% N content.

3	10	1	7	2	5	9	6	4	8
1	7	8	5	4	3	6	9	2	10
1	2	7	5	8	10	3	6	4	9

5	6	9	8	7	10	3	7	2	4
8	2	10	7	1	4	5	3	9	6
8	4	9	7	1	5	10	3	2	6

Figure 1. Random distribution of treatments according to their numbering in the experimental site. Each number represents an experimental unit of a treatment (10 treatments with 6 repetitions = 60 experimental units).

Figure 2. a) Treatment mixture and b) wrapped treatments.

Study variables

At the beginning of the process, four sugarcane filter-pressed mud, bagasse, ash, and straw samples were collected for their physical and chemical characterization. Every 30 day, samples were collected to analyze the pH, MO, NH_4^+, NO_3^- variables (three repetitions per variable) (Figure 3). They were dried in the shade, ground, and sieved using a 2 mm mesh for their analysis according to Pérez *et al.* (2011) and the NMX-FF-109-SCFI (2008). Two-hundred seventy analyses were carried out.

Figure 3. Sampling process: a) sampling and b) sample labelling every 30 days.

Statistical analysis

The study variables were subjected to an analysis of variance with a 5×2 factorial arrangement, considering the mixture factors (100% sugarcane filter-pressed mud, 100% sugarcane filter-pressed mud + 0.5% N, 75% sugarcane filter-pressed mud + 25% bagasse, 70% sugarcane filter-pressed mud + 20% straw + 10% ash, and 60% sugarcane filter-pressed mud + 30% straw + 10% ash), aeration times (7 days and 14 days), and six repetitions. Variables with significant differences between treatments were subject to Tukey's multiple comparison test ($P \leq 0.05$) using the SAS 9.2 statistical software.

RESULTS AND DISCUSSION

Physical and chemical characteristics of sugarcane filter-pressed mud compost

Table 2 shows the analysis of variance of the physical and chemical characteristics of the various compost mixtures and aeration times under review. Neither the different mixtures nor the aeration times affected the pH. Organic matter content (OM, %) was significantly affected by different compost mixtures. Nitrates (NO_3^-, ppm) were not affected by any factor. Ammonium (NH_4^+, ppm) showed significant differences regarding the compost mixture factor.

Hydrogen potential (pH)

No significant pH differences were observed for the mixture, aeration times, and interaction factors (Table 2). The mean pH value of the treatments fluctuated between 8.4 and 8.9, which are normal values for organic substrates (Hernández *et al.*, 2013). Torres-Lozada *et al.* (2021) also reported an 8.13 pH in a sugarcane filter-pressed mud-based compost. The presence of sugarcane filter-pressed mud treated with lime ($CaCO_3$) —as is the case of the sugarcane filter-pressed mud produced at ISR during this study— increased pH values above 8; therefore, it is useful to alkalinize the pH of acid soils (Dotaniya *et al.*, 2016). Mendez *et al.* (2011) observed that composts containing a higher proportion of sugarcane filter-pressed mud in relation to bagasse have a higher pH than those cases when there is more cane bagasse than sugarcane filter-pressed mud. According to the NMX-FF-109-SCFI-2008, the pH values of compost must range from 5 to 9.

Mendez *et al.* (2011) observed that adding ammonia to sugarcane filter-pressed mud and bagasse mixtures caused a pH decrease in treatments with ammonia, while treatments without ammonia maintained a steady pH value of 7.70. Such was the case of the 100% sugarcane filter-pressed mud + 0.5% N mixture in this study, which recorded the lowest pH values (8.43), in contrast to the 100% sugarcane filter-pressed mud treatment, at both aeration time levels. Towards the end of the composting process, pH drops as a result of the formation of low-molecular weight organic acids.

Furthermore, Official Mexican Standard NMX-AA-180-SCFI-2018 states that the pH of the finished composts ranges from 6.7 to 8.5, which seems to be the case only of the ammonium-added treatment.

Organic matter (OM, %)

The organic matter (OM, %) content of different compost mixtures ranged from 28.03 to 33.89%, values that are considered to be within the optimum range (20-50% OM) established by the Official Mexican Standard NMX-FF-109-SCFI-2008. Our results are also in line with the organic matter content of organic substrates of sugarcane filter-pressed mud reported by Martínez *et al.* (2021) and are lower than those reported by Hernández-Melchor *et al.* (2008) for the sugarcane filter-pressed mud-based compost (58.6% OM). At 7 days of aeration, the 75% sugarcane filter-pressed mud + 25% bagasse treatment, with an average 33.89% OM, showed the highest pH values compared to the other treatments (Table 3). Treatments including a certain proportion of cane bagasse have a higher OM percentage regarding sugarcane filter-pressed mud-based treatments, since bagasse mainly contains organic compounds such as cellulose, hemicellulose, and lignin (Méndez *et al.*, 2011; Salgado *et al.*, 2013).

Nitrates (NO_3^-, mg kg^{-1})

NO_3^- values ranged from 5 to 13.15 ppm. According to the CCQC criteria (2001), <100 ppm values are found in very mature composts. For their part, Méndez *et al.* (2011)

Table 2. Mean squares of ANOVA for the chemical properties of the different compost mixtures and aeration times under review.

Factor of variation	GL	Mean Square			
		pH	MO (%)	NO_3^- (mg kg^{-1})	NH_4^+ (mg kg^{-1})
Mixtures (M)	4	0.42 NS	52.83**	19.02 NS	584.22**
Time (T)	1	1.4×10^{-3} NS	7.23 NS	0.20 NS	53.92 NS
M*T	4	0.11 NS	8.86 NS	129.45 NS	19.69 NS
Error	80	0.30	8.10	183.8	45.54
CV (%)		6.3	9.21	48.91	58.98

C=cycle, V=variety, SS=soil subunit SS>V=nested factor (V within SS), GL=degree of freedom, *=P<0.05, **=P<0.01, NS=not significant.

Table 3. Tukey's multiple comparison test of the organic matter content (OM, %), pH, NO_3^- (ppm), and NH_4^+ (ppm) of the various compost mixtures and aeration times under review.

Treatment	Compost mixtures	Aeration time (days)	pH	OM (%)	NO_3^- (ppm)	NH_4^+ (ppm)
1	Sugarcane filter-pressed mud 100%	7	8.81a	31.14ab	6.92a	11.29bc
2	Sugarcane filter-pressed mud 100% + 0.5% N	7	8.43a	30.62ab	12.02a	20.62ab
3	Sugarcane filter-pressed mud 75% + bagasse 25%	7	8.87a	33.89a	11.38a	9.77c
4	Sugarcane filter-pressed mud70% + 20% straw + 10% ash	7	8.52a	28.03b	8.57a	10.35bc
5	Sugarcane filter-pressed mud 60% + 30% straw + 10% ash	7	8.8a	29.36b	6.79a	9.06
6	Sugarcane filter-pressed mud100%	14	8.78a	31.62ab	13.15a	10.64bc
7	Sugarcane filter-pressed mud 100% + 0.5% N	14	8.43a	31.86	8.37a	22.16ab
8	Sugarcane filter-pressed mud 75% + bagasse 25%	14	8.71a	32.23ab	5a	5.87c
9	Sugarcane filter-pressed mud 70% + 20% straw + 10% ash	14	8.78a	28.67b	7.33a	7.62c
10	Sugarcane filter-pressed mud 60% + 30% straw + 10% ash	14	8.7a	31.49ab	11.36a	7.04

* Results with different letters are significantly different ($P \leq 0.05$). The values represent the means of each treatment.

recorded that NO_3^- content diminished as cane bagasse increased in the substrate, while the content increased in treatments with a higher proportion of sugarcane filter-pressed mud. These results are explained by the almost null nitrate $\left(NO_3^-\right)$ formation during the initial stages of composting. NO_3^- is only released until the substrate has got past the thermophilic phase and reached a mesophilic range, because that is the moment when organisms emerge transforming NH_4^+ into NO_3^- (Montoya-Jasso *et al.*, 2021): Therefore, we can infer that the changes that occur during the thermophilic phase —such as the decrease in pH and increase in EC— favor the increase of N-ammoniacal and nitrates (Méndez *et al.*, 2011), as was the case of the ammonium-added treatments with the lowest pH values.

Ammonium (NH_4^+, mg kg^{-1})

Ammonium $\left(NH_4^+\right)$ showed significant differences only in the mixing factor (Table 2). According to the criteria established by CCQC (2001), the NH_4^+ concentration of a compost depends on its degree of maturity: >500 ppm means an immature compost; between 100-500 ppm, a mature compost; and <100 ppm, a very mature compost. The third category corresponds to the composts studied in this research. Our results pointed out that adding ammonia to the 100% sugarcane filter-pressed mud + 0.5% N treatments in both aeration times achieved the highest values of NH_4^+ compared to the treatments without additional ammonia (Méndez *et al.*, 2011; Torres-Lozada *et al.*, 2021).

In our study, composts are classified as very mature stabilized organic materials (CCQC, 2001). The lower NH_4^+ content recorded in the mixture with bagasse was the result of the transformation of ammonium and the slow degradation of residues with a high C:N ratio, such as sugarcane bagasse (Méndez *et al.*, 2011; Salgado *et al.*, 2013; Martínez *et al.*, 2021). The combination of sugarcane filter-pressed mud and bagasse with 90-day composting showed a stable carbon-nitrogen (C:N) ratio, as well as a low amount of ammoniacal nitrogen $\left(NH_4^+\right)$. Consequently, the plant has enough nitrate available (Quiroz and Pérez, 2013), as was the case of the 75% sugarcane filter-pressed mud + 25% bagasse treatment at 7 days of aeration time (Table 3).

CONCLUSION

Aeration time did not show significant differences regarding the quality parameters of composts. All treatments had optimal pH and OM levels. The 100% sugarcane filter-pressed mud and 100% sugarcane filter-pressed mud + 0.5% N treatments obtained the highest nitrate $\left(NO_3^-\right)$ values (13.12 and 12.02 ppm, respectively), as well as high ammonium $\left(NH_4^+\right)$ content (22.16 and 11.29 ppm, respectively). All the mixtures were mature composts and suitable for replacing chemical fertilization.

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AP AGRO PRODUCTIVIDAD

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Physical and chemical properties of soils irrigated with vinasses for the cultivation of sugarcane (*Saccharum* spp.) in the central region of Veracruz, Mexico

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ABSTRACT

Objective: To evaluate the physical and chemical properties of a soil irrigated with vinasse for two years (+V), compared with a soil without vinasse (−V) application.

Design/Methodology/Approach: The following parameters were evaluated for both agroecosystems: texture, pH, electrical conductivity (EC), organic matter (OM), phosphorus (P), potassium (K), nitrate $\left(N\text{-}NO_3^-\right)$, and total nitrogen (TN). The evaluation followed the standardized methods established in NOM-021-SEMARNAT-2000.

Results: There were no significant changes ($p>0.05$) in the soil's pH and electrical conductivity. However, the application of vinasse significantly increased ($p<0.05$) the concentrations of TN (1.52%), K (112.00 mg L^{-1}), and OM (4.52%) in relation to soils −V (0.78%, 25.60 mg L^{-1}, 7.40 mg L^{-1}, and 2.75%, respectively).

Study Limitations/Implications: There are few studies about the contributions and the physical and chemical effects of soil irrigation with vinasses in the State of Veracruz.

Findings/Conclusions: Even though vinasse had a positive effect on the physical and chemical characteristics of the soil, the mineral fertilization program must be reformulated to increase the K and P concentration in soils with silt loam texture. In addition, we recommend adjusting and normalizing the dose of N that vinasse can provide to complement conventional fertilizers.

Keywords: Soil fertility, Sugarcane, Vinasses.

INTRODUCTION

Sugarcane has been cultivated in Mexico for over 450 years. At present, Mexico is the sixth world sugarcane producer, with an annual yield of 61 million tons. Fifteen Mexican states (261 municipalities) are involved in this activity. Together, they generate 11.6% of the primary sector value, equivalent to 0.4% of the gross domestic product (Sentíes-Herrera *et al.*, 2014; Pérez-Sánchez, 2017).

Image by Joseph Mucira at Pixabay

The State of Veracruz has the greatest number of sugarcane mills (18 out of 50) and the largest cultivated area (284,000 ha) in Mexico (Pérez-Sánchez, 2017). It has the highest sugarcane production in the country. The four main cultivated varieties are CP 72-2086, MEX 68-P-23, MEX 69-290, and MEX 79-431, which contribute 42.82% to the national sucrose production and have an annual yield of approximately 110 ton ha^{-1} per variety (SAGARPA, 2016; SIAP, 2020; Vera-Espinosa *et al.*, 2016).

Some studies conducted in the central region of the state show that the La Gloria and San Nicolás sugar and alcohol mills produce nearly 33 million liters of bioethanol from sugarcane molasses distillate (Becerra-Pérez, 2009). It is estimated that for each liter of ethanol produced in the sugarcane agro-industry, 13 L of vinasse are generated (de Mello-Prado *et al.*, 2013), which results in an annual production of around 430 million L of this byproduct. Vinasse is a viscous liquid of acidic pH with a high electrical conductivity, a high chemical oxygen demand, and a high concentration of organic matter and nutrients, especially K, N, and P (España-Gamboa *et al.*, 2011).

The high volume of vinasses generated by the sugar industry has led to the research into various forms that the nutrients they contain can be used. In the central region of Veracruz, producers use vinasses to supplement conventional (inorganic) fertilization through irrigation. Although this practice favors crop yields, its implementation has not been welcomed by the general population. Blaming its unpleasant smell and occasional undesirable effects after its application, 68% of producers in Veracruz reject the use of vinasse on crops (Quiroz-Guerrero *et al.*, 2011). This social perception and its concomitant negative attitude could be related to the lack of a comprehensive management of vinasse as a byproduct of the sugar industry. They could also be the result of a lack of training and systematic research programs in the field, as well as of the lack of adequate assessments of the effects of vinasses on the soil's physical and chemical properties.

Consequently, the objective of this work was to determine the physical and chemical properties of a representative soil of the sugarcane agroecosystem in the central region of Veracruz (+V), comparing it with a control soil (−V). Reference properties are indicators of soil quality. Therefore, this study will lay the foundations for regional producers to establish, in the mid-term, adequate fertilization doses, subsequently optimizing the agronomic efficiency and effectiveness of N, P, and K in the production and quality of sugarcane.

MATERIALS AND METHODS

Description of the study area

The study was conducted in the municipality of Paso de Ovejas, Veracruz, Mexico (19° 08' and 19° 22' N; 96° 20' and 96° 38' W), located in the Microregion of Priority Attention (MAP) of the Colegio de Postgraduados Campus Veracruz (Figure 1). The municipality has 97 localities with a total area of 387.8 km^2, 263.3 km^2 of which are used for agriculture. Warm sub-humid climate predominates in the region (average humidity of 61%), with a temperature of 24-25 °C, and an average annual rainfall of 1,500 mm. Topographically, the altitude of region ranges from 10 to 400 m and is part of the sugarcane area of the State of Veracruz, with a cultivation area of 3,500.8 ha and an annual harvest of 357,076.5 tons (SIM, 2019).

Treatments and experimental design

Two similar plots located close together were selected for this study. Both have been cultivated with sugarcane for the last 30 years; one of them has a history of vinasse irrigation (+V) and the other one does not (−V) (Figure 1). The soil −V was treated twice a year with conventional fertilizers. The first application (NPK 20-10-20 fertilizer) was carried out after cutting. The second fertilization was applied with urea (46-00-00), forty days after the first fertilizer was applied. The vinasse-irrigated soil (+V) followed the same conventional fertilization arrangement as the soil −V; however, from 2018 onwards, vinasse was diluted in the irrigation water once a year, using the following method: five irrigations of 20 m^3 $plot^{-1}$ were carried out, with a one-hour interval between applications. Vinasse was applied after cutting and the conventional fertilizer was applied for the first time 20 days later. The sugarcane variety cultivated in the soil without vinasse (−V) was ColMex 95-27; meanwhile, the variety used in the soil with vinasse (+V) was CP 72-2086, an early maturing variety. The sampling, preparation, and analysis of the samples were conducted following NOM-021-SEMARNAT-2000 (SEMARNAT, 2000).

Determination of the physical and chemical parameters of soil samples

Physical and chemical parameters were determined for both soil samples. Phosphorus (P), potassium (K), and nitrate $\left(N\text{-}NO_3^-\right)$ were quantified by spectrophotometry using

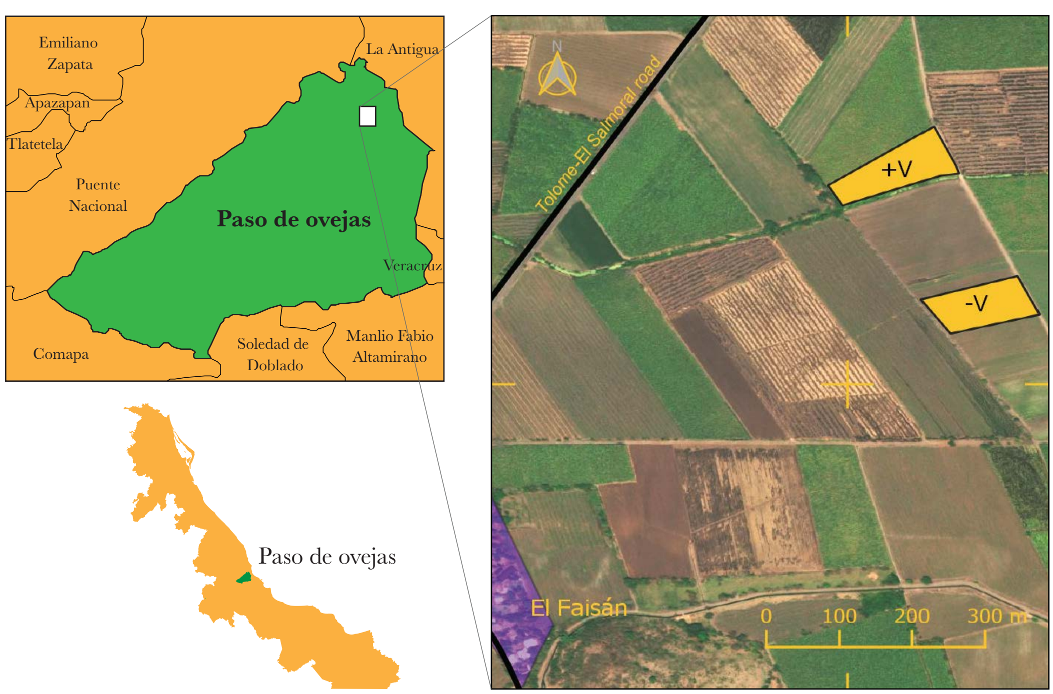

Figure 1. Location of the study plots, with (+V) and without vinasse application (−V), within the municipality of Paso de Ovejas, Veracruz, Mexico (QGIS 3.0).

a HI 83225 multi-nutrient analyzer (Hanna Instruments). According to the methods standardized in NOM-021-SEMARNAT-2000, the texture was determined using the Bouyoucos method; organic matter (OM) by wet combustion (Walkley and Black); and total nitrogen (TN) by the traditional Kjeldahl method. Potential hydrogen (pH) and electrical conductivity (EC) were measured using a potentiometer (Hanna Instruments) and a HI 2300 conductivity meter (Hanna Instruments), respectively.

Statistical analysis

An analysis of variance (ANOVA) was conducted for all assessed physical and chemical variables. A Tukey's test with a significance level of $p<0.05$ was carried out when significant differences were found between the means of both soils. Analyses were conducted using the InfoStat software.

RESULTS AND DISCUSSION

Table 1 presents the average values of the main physical and chemical properties determined in soils with (+V) and without (−V) a history of vinasse irrigation.

Applying vinasse did not modify the soil's physical properties. The analyzed soils had clay loam (−V) and silt loam (+V) textures. Although the use of soils with loam clay textures is recommended for the cultivation of sugarcane, producers in the central region of Veracruz have grown sugarcane efficiently in different soil types with no negative effects on production yields (Aguilar-Rivera, 2014). After the cultivation period, the sugarcane yield per hectare recorded for the cultivated varieties matched the potential mean values for the region (130 t ha^{-1} for ColMex 95-27 in soil −V and 105 ton ha^{-1} for early maturing CP 72-2086 in soil +V) (SAGARPA, 2016). Yield values are mentioned as a point of reference, since the relation between yields and the changes resulting from the application of vinasse was not measured.

The average pH of soils −V and +V remained in the neutral range (7.06-7.00, respectively), which indicates that the vinasse application combined with a conventional fertilization (commercial chemicals) during two consecutive years did not alter this

Table 1. Physical and chemical properties of sugarcane soils with (+V) and without a history of vinasse application (−V). Values belong to the mean ± standard error.

Parameter/Nutrient	Soil without vinasse irrigation (−V)	Soil with vinasse irrigation (+V)
pH (u. pH)	7.06 ± 0.03^{a}	7.00 ± 0.31^{a}
Electric conductivity (dS m^{-1})	0.435 ± 0.040^{a}	0.544 ± 0.085^{a}
Texture	Clay loam	Silty clay
Organic matter (%)	2.75 ± 0.10^{b}	4.52 ± 0.13^{a}
Phosphorus (P) (mg L^{-1})	5.20 ± 0.31^{a}	3.50 ± 0.14^{b}
Potassium (K) (mg L^{-1})	25.60 ± 6.73^{b}	112.00 ± 15.92^{a}
Nitrate $\left(N\text{-}NO_3^-\right)$ (mg L^{-1})	7.40 ± 1.24^{a}	1.75 ± 1.02^{b}
Total nitrogen (%)	0.78 ± 0.02^{b}	1.52 ± 0.05^{a}

Different letters in the same row indicate significant differences ($p<0.05$).

parameter. Similar trends regarding the pH's attenuation capacity were observed in other studies that applied 150, 300, and 450 m^3 vinasse ha^{-1} doses in sugarcane crops (Del Pino *et al.*, 2017). Likewise, Jiang *et al.* (2012) point out that no acidification was observed after three years of uninterrupted vinasse application in sugarcane crops. Nevertheless, the pH values in both studies were lower (5.1-5.4 and 4.89-4.93, respectively) than the results obtained in this work.

Although no pH variations were found in the soil +V, further research is needed to determine whether or not vinasses have a long-term effect on the pH of soils in the sugarcane agroecosystems of the central region of Veracruz. This would help to avoid soil acidification, since changes in the pH are more likely to become evident during longer application periods.

The differences between both soils regarding EC were not significant ($p>0.05$) (Table 1). The values found (0.435 dS m^{-1} in soil −V and 0.543 dS m^{-1} in soil +V) do not indicate salinity problems, since the saline effect is usually negligible when EC values are under 1 dS m^{-1} (NOM-021-SEMARNAT-2000). According to Soobadar (2014), for sugarcane yields to undergo a 10% decrease, EC values would have exceeded 1.7 dS m^{-1}.

In this study, the low EC was also reflected on the content of primary nutrients (*e.g.*, P and K). The P concentration fluctuated between 3.50 mg L^{-1} (+V) and 5.20 mg L^{-1} (−V); these levels are considered low for sugarcane cultivation. As was expected, the vinasse significantly increased ($p<0.05$) the concentration of K in relation to the control soil (−V). In spite of this, the values of K in soils +V (118 mg/L) fell below the requirements for the development of sugarcane in silty clay soils (156-295 mg L^{-1}). These results showed a deficiency in the P and K concentration that the plant can absorb (Del Pino *et al.*, 2017).

The sugarcane crop has a high nutrient demand, particularly of N (58.8-200 kg N ha^{-1}) P (33.3-172 kg P ha^{-1}), and K (86.4-417.5 kg K ha^{-1}) (Salgado *et al.*, 2006). The unavailability of these nutrients can cause a drop in sucrose contents (Salgado *et al.*, 2006; Guerrero-Peña *et al.*, 2017).

These results suggest that the mineral fertilization program should be reformulated to satisfy the P and K demand of the sugarcane soils in the central region of Veracruz, particularly during the second fertilization, when the applied fertilizer is based exclusively on urea (40N-00-00 composition).

Meanwhile, a positive effect was observed on the OM recovery when the soil was treated with vinasse. The concentration of OM in the soil +V was 4.52 mg L^{-1} (high level), a significantly higher value ($p<0.05$) than the control soil −V (2.75 mg L^{-1}, medium level). Some studies have shown that the uninterrupted application of low vinasse doses during periods of up to three years (as is the case of this study) can improve the soil's physical conditions —such as the compaction state (bulk density decreases while porosity increases)—, help to attenuate the soil's acidity (Jiang *et al.*, 2012, Sánchez-Lizárraga, 2018), and foster a greater nutrient mobilization (Neves *et al.*, 1983). As shown in Table 1, vinasse increased the percentage of OM and nutrients in the soil; both elements improve the crop's quality and fertility.

Regarding TN, vinasse is a byproduct with a low N content (0.1 g m^{-3}-0.73 g m^{-3}) (Carvalho *et al.*, 2013; Silva *et al.*, 2014); however, when a 100 m^3 ha^{-1} dose is added, the N content can reach 10-73 kg N ha^{-1} rates (Silva *et al.*, 2015). Therefore, the contribution of vinasses, combined with conventional fertilization, reaches significant N levels. In this study, vinasse was a significant source of N ($p<0.05$), reaching levels well above those required by the crop. Therefore, an appropriate nitrogen fertilization program should be established in order to avoid the loss of this nutrient through volatilization, lixiviation, or denitrification processes.

The concentration of N in soils +V (1.52%) was two times higher than in soils −V (0.78%) that were treated only with conventional fertilizers. In spite of this contribution, the N released by vinasse seems to have been slow, probably due to the mineralization of different types of organic nitrogen (Del Pino *et al.*, 2017). Alotaibi and Schoenau (2012) estimate that approximately 20% of the total N in vinasse is found as ammonium, a cation that, once absorbed, is immediately assimilated by sugarcane. However, the soil's neutral pH conditions suggest that part of the ammonium becomes ammonia. This transformation seems to indicate that volatilization could be the partial cause of the loss of ammonium in soil +V (CONADESUCA, 2008; Silva *et al.*, 2015). This is directly related to the low nitrate concentration (1.75 mg L^{-1}) recorded in relation to the soil −V, where most N converted into nitrate (7.40 mg L^{-1}) through nitrification.

Finally, a residual effect from vinasse benefits the crop directly. It is a viable strategy to recycle nutrients and consequently reduce dependency on conventional fertilizers.

CONCLUSIONS

Vinasse combined with conventional fertilizers has several potential advantages for the soil's properties, such as an increase in the levels of K, TN, and OM. The application frequency did not cause salinity problems in soils with a silt loam texture. However, the fertilization scheme should be normalized to adjust the P and K concentration to the optimal levels for the cultivation of sugarcane. Applying 100 m^3 ha^{-1} doses of vinasse was a viable alternative for the supplementary conditioning and fertilization of the soil, since it provides a source rich in OM, water, and nutrients that meet the nutritional requirements of sugarcane at a low cost. Nevertheless, it is important to adjust the OM and TN levels in order to prevent their loss by volatilization, lixiviation, or denitrification processes which could contribute to the increase greenhouse gas emissions (particularly CO_2 and N_2O) or have a negative impact on superficial water and groundwater sources.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Housing economic valuation in the metropolitan area of Puebla, Mexico

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ABSTRACT

Objective: The purpose of this research was to identify the sale value of homes in the metropolitan area of the city of Puebla, using the hedonic price technique to determine a model that takes into consideration relevant variables that explain the housing prices.

Methodology: An overall problem is land mismanagement. The location of the homes can affect their price. A hedonic price model helps to determine the relative importance of the variables in the final price. This information provides an initial perspective to the people who are interested in acquiring a home in the study area. The model was built based on the information collected from 182 properties and it was analyzed using the SPSS software version 28.0.0.0.

Results: The most significant variables that determine housing prices, which presented a multiple valuation coefficient of 64.3%. The effect of the variables on the housing value was determined by means of elasticities.

Study Limitations: There were no limitations for this report.

Conclusions: The following variables were significant: new home, number of bathrooms, built-up area, private security, distance to the downtown of Puebla, and distance to a park.

Keywords: Housing price, Hedonic regression model, Puebla.

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INTRODUCTION

The population has grown exponentially in the last 300 years and technological progress has modified the environment to satisfy human needs, based on the exploitation of natural resources that a large part of the population considered inexhaustible (Barbieri & Fogel, 2005; Labandeira Villot *et al.*, 2001). In recent years, the housing sector in Mexico has been boosted by lower interest rates and an increase in the credits granted by both private and government entities. However, all of the country's housing needs are yet to be met. Another important aspect is that the employment instability faced by the new generations means that they lack feasible possibilities of buying a home (Tirole, 2008).

Image by Brigitte Werner at Pixabay

Mexico's population policy is based on the reform of the Ley General de Población of 1974. Since that time, the population has experienced major quantitative and qualitative changes (*e.g.*, the growth of urban areas in national territory). These changes have shown the need for a comprehensive understanding of urban development, based on a sustainable development approach to urban areas (CONAPO, 2014; UN-Habitat, 2018). Cities were not prepared to receive these migration flows and there was not a sufficient supply of suitable and accessible land for housing. Consequently, millions of Mexicans settled in areas that were unsuitable for housing construction. The poor location of homes can be explained by the concept of spatial capital, which allows assessing physical accessibility to goods and services (Coneval, 2018). Spatial capital is a type of urban resource internalized by the individual resulting from the combination of objective material conditions (graphics, design, infrastructure availability, accessibility, and connectivity) and subjective socio-cultural conditions (how they use and make the spaces and means of transport their own) (Apaolaza *et al.*, 2016; Coneval, 2018).

The metropolitan area of Puebla faces important challenges as a consequence of the use of high agricultural-value land and other areas adjacent to the city, resulting from land management and water demand. These problems have been caused by the absence of intermunicipal agreements on issues such as solid waste management, air pollution, transportation, and changes in land-use. The real estate market situation is influenced by several factors. The analysis of the socioeconomic, environmental, and accessibility aspects enables the identification of which of them have a greater impact on the configuration of the sale value and how it is affected by certain actions —such as urban planning, equipment, infrastructure, crime, etc.

The overall objective of this research is to analyze, through the hedonic price technique, the relationship between variables that may interfere with the property sale value in seven municipalities of Puebla's metropolitan area. Such variables include distance to the downtown, a nearby park or a hospital, and the number of crimes. The following hypotheses are formulated: a) the longer the distance from parks, the lower the sale value and b) the greater the number of crimes, the lower the sale value.

MATERIALS AND METHODS

Puebla is one of the most important states of Mexico, not only in the economic sphere —to which it contributes 3.4% of the domestic GDP—, but also in population terms —since about 4.8% of the country's inhabitants live in the state.

The accelerated urban expansion of the metropolitan area agglomeration of the city of Puebla between 1976 and 2005 —and the resulting transformation of agricultural and forest land into urban land— has contributed to the deterioration of the environment and natural resources (Hernández-Flores *et al.*, 2009).

Puebla, as well as its metropolitan area, has undergone a continuous growth and it concentrates the largest population in the state. The study focused on 7 municipalities that have a population of approximately 2.15 million inhabitants (INEGI, 2010). These municipalities are: Amozoc, Coronango, Cuautlancingo, Ocoyucan, San Andrés Cholula, San Pedro Cholula, and the city of Puebla itself.

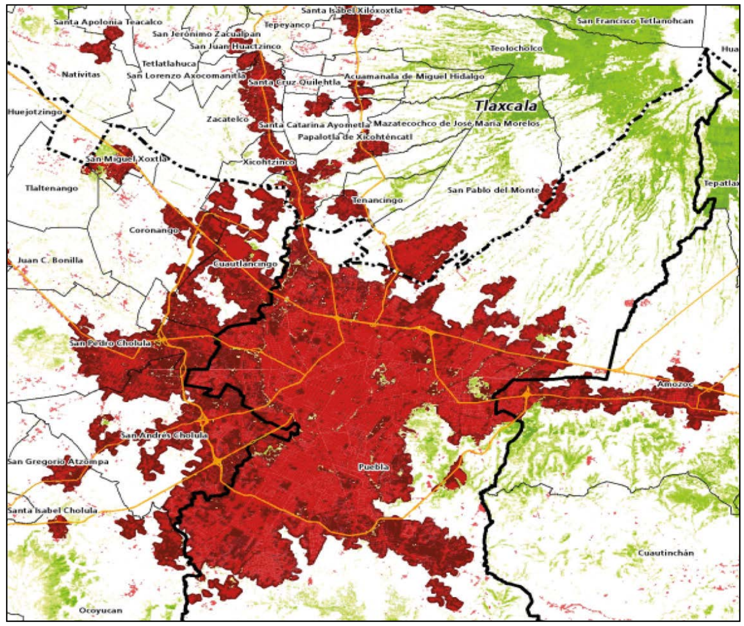

Figure 1. Puebla metropolitan area (ONU-Habitat, 2018).

Table 1. General characteristics of some municipalities of Puebla's Metropolitan Area.

Municipality	Urban area (Km^2)	Population	Density	
			Inhab/km^2	Homes/km^2
Amozoc	12.6	138,214	84	2151
Coronango	3.1	40,949	1049	2942
Cuautlancingo	33.2	112,235	2647	929
Ocoyucan		25,720	214	
Puebla	168.6	1,576,259	3025	2554
San Andrés Cholula	21.9	137,290	1849	1723
San Pedro Cholula	12.2	128,032	1684	2517

Source: developed by the authors based on data from ONU-Habitat (2018).

Model and variables

Hedonic prices

The hedonic price technique is based on the fact that the merchandise offered in a given market has homogeneous characteristics and that each merchandise is either made up of a series of components that determine its value or is based on the relationship that exists between the consumer preferences and the characteristics of real estate (Lancaster, 1966).

People buy homes from the market with the intention of increasing their well-being, because they consider that these goods have a set of characteristics that satisfy some of their needs, as a consequence of their use value. Many goods do not have a single use value, given that they satisfy several needs at the same time and can be classified according to their physical characteristics. In the case of housing, some characteristics may be: social and spatial environment or its environmental and geographical location.

Their combination determines the amount that the user is willing to pay for the property (Fitch Osuna *et al.*, 2013).

Variables

We seek to develop a model that can test the hypotheses regarding the sale value of homes in the metropolitan area of Puebla, taking into consideration that the combination of variables determines the value. The economic valuation method used is the hedonic price technique. In this case, the method breaks down the value of a property based on its characteristics and development aspects. The standard specifications of this model are based on Sobrino-Figueroa (2014).

$$P_1 = \alpha + X_1\beta + \varepsilon_1$$

Where P_1 represents the value of the house, α is the coefficient, X_1 represents a vector of attributes or physical and spatial characteristics of N houses in Puebla's Metropolitan Area, β is the vector associated with each characteristic, and ε is a vector of errors, independently and identically distributed for all N houses. The proposed model is based on the fact that user preferences regarding the attributes or characteristics in question are identical for all individuals (Brueckner & Colwell, 1983).

The data used belongs to 61 neighborhoods distributed in 7 municipalities of the metropolitan area of the city of Puebla (Table 1). The sale value and the average size, as well as the physical characteristics of the housing, were obtained from a real estate company that operates in Puebla's metropolitan area, as well as from Propiedades.com, tuhogarmexico.com, Inmuebles24, and Lamudi (2020).

Vector is made up of other characteristics of the homes that are taken into consideration to explain the value (Table 2). One of Google Maps[5] tools was used to determine the distance (km) from each neighborhood to a hospital, to Puebla's downtown, to parks, and to a shopping center. Another important aspect that is taken into account is the number of crimes per 100 thousand inhabitants, based solely on data provided by the Fiscalía General del Estado de Puebla (2020) —therefore the actual data may have been underestimated (although determining the appropriate number is not the objective of this research). The effects that this variable would produce are reverse, regardless of the type of crime that is committed (whether it impacts life, well-being, personal freedom, sexual freedom and safety, property, family, society, etc.).

RESULTS AND DISCUSSION

The ordinary least squares (OLS) method was applied to estimate the best model. This method has been used by Jansson and Axel (2000) and Nuñez C. and Schovelin S. (2002) for functional relationships that have proven to be appropriate for these kinds of problems.

[5] The Google Maps tool is used to determine the distance in kilometers from each home to the point of interest (DZP, DP, DH, DCC).

Table 2. Variables used in the model.

Variable	Denomination
New house	Cnu
Number of bathrooms	Bañ
Number of bedrooms	Recam
Parking lot	Est
Built-up area (m^2)	Cons
Private security	SP
Distance to Puebla's downtown (km)	DZP
Distance to a park (km)	DP
Distance to a hospital (km)	DH
Distance to a shopping center (km)	DCC
Population density (inhab/km^2)	DenP
Homicides per 100,000 population	Hom
Crimes per 100,000 population	Del
Criminal events (Hom + Crim)	EveDel

Source: developed by the authors based on the variables used.

The coefficients of the variables are obtained through the OLS which, in their turn, are used to carry out the significance tests. The available information is used to develop the best model.

The model presented is the result of discarding variables that did not provide substantial information. To reach this determination, a Student's t-Test was carried out to analyze the values and to determine their significance. Subsequently, the model shown in Table 3 was chosen.

Table 3. Result of the hedonic price model for homes in the metropolitan area of Puebla with significant variables.

Variables	Coefficients	t
(Constant)	14.016	5.537
Cnu	0.123	1.699
Bañ	0.054	1.745
Cons	0.004	8.043
SP	0.174	2.622
DZP	−0.018	−2.411
DP	−0.047	−3.42

Model Summary

Model	R squared	R squared adjusted	Standard error of the estimate
1	0.643[a]	0.625	0.38549

[a] Predictors: (Constant), Del, Hom, Bañ, SP, Cnu, DP, Cons, DZP, DenP.

Source: developed by the authors based on the results of the regression model.

Based on Table 3, the functional form of the model can be developed. The model would be represented as follows:

$$P = 14.016 + 0.123Cnu + 0.054Bañ + 0.004Cons + 0.174P - 0.02DZP - 0.047DP$$

The model accounts for 64.3% of the variation in the sale value of homes, based on the independent variables included in the hedonic price model for the metropolitan area of Puebla. The variables that meet the significance criteria of the hedonic price model are analyzed next, taking the price elasticity of each variable as a reference.

Table 4 shows the price elasticities of the model. All the variables have an inelastic behavior: a 1% change in the variables will have a smaller effect in the sale price of the housing properties.

Based on the elasticity, the price for new homes (Cnu) will be 0.07% higher than for other homes with the same characteristics.

The price elasticity for the number of bathrooms (bañ) shows that increasing this variable by one unit increases the value of the house by 0.15%.

With respect to the elasticity obtained, the built-up area (Cons) points out that a one-unit increase in the built-up area increases the value of the house by 0.82%.

Meanwhile, based on the elasticity obtained, private security (SP) in the area where a property is located increase the sale value by 0.09% regarding homes that lack the service.

The effect of the distance to Puebla's downtown (DZP) means that, for each additional kilometer, the housing prices decreases by 0.21%.

Finally, the distance to a park (DP) points out that each additional kilometer decreases the value of the house by 0.19%.

Brueckner and Colwell (1983) mention that implementing this type of mathematical model to explain the value of housing is a valid measure, since it recognizes the existence of multiple attributes. Housing in the metropolitan area of Puebla has characteristics that have been used in analyses such as the one carried out by Murrieta and Lagunas (2012). They take into account the characteristics proposed in this model and also determine that users prefer housing with several bedrooms and bathrooms and that is near a park and at a certain distance from downtown, as shown by the results obtained in the previous section. The social environment is also an aspect that has a major impact on the sale value

Table 4. Price elasticities of the model.

Variable	Price elasticity
Cnu	0.07
Bañ	0.15
Cons	0.82
SP	0.09
DZP	−0.21
DPCA	−0.19

Source: developed by the authors based on the results of the regression model.

of housing properties: the variable that covers private security in the housing area is an important factor.

According to the model, the housing price depends on the distance to certain points. In other words, the distance between the home and certain points of interest for the individuals has a direct impact on the price, whether it is the downtown or new centers or poles of attraction. This aspect is also highlighted by Lara Pulido *et al*., (2016) and Murrieta and Lagunas (2012).

CONCLUSIONS

Three conclusions can be drawn from the analysis of the results. In the first place, the proximity to Puebla's downtown generates the greatest value for consumers: the greater the distance, the lower the value. In second place, the social environment is also an important aspect. The results of this model generate a negative effect, as stated in the hypothesis regarding the number of crimes. An increase in the number of crimes generates a decrease in the housing value. However, a statistical analysis reports no significant level; therefore, we decided not to use it since it does not make a significant contribution to the model. However, private security in the area where the property is located does have a positive impact on its value, which is related to the social environment. In third place, the value of housing located in the metropolitan area of Puebla has an important relationship with its surrounding environment. To have a higher value, it must be close to the downtown, not far from a park, and have an average of just over 200 square meters of built-up area.

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AP AGRO PRODUCTIVIDAD

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Estimating biomass in grasslands through traditional methods and the use of drones in the State of Chihuahua, Mexico

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ABSTRACT

Objective: To evaluate three biomass estimation methods (Unmanned Aerial Vehicle (UAV or drone), ceptometer, and canopy height), comparing them to the quadrant method in an arborescent tufted grassland in the state of Chihuahua.

Methodology: The study was conducted in Teseachi, Namiquipa, in october 2020. We located thirty random points. The first biomass estimation method used was UAV. Once the drone flights were completed, the quadrant was placed and the coordinates were determined. We carried out nine readings using a ceptometer and obtained an average. Subsequently, we measured the average canopy height. Finally, all forage within the quadrant was cut at ground level and packed for laboratory analysis. The Agisoft Metashape software was used to process the SfM of the aerial images, using nine sampling points, applying the NGBDI vegetation index, and calculating the average pixels of a 3×3 m moving window. A simple linear regression model was used to analyze the data with the R Project software, version 4.0.3.

Results: The simple linear regression model showed an R^2 of 0.62 ($p<0.01$), 0.55 ($p<0.001$), and 0.48 ($p<0.001$), for UAV, ceptometer, and canopy height, respectively.

Study Limitations: There were no limitations for this report.

Conclusions: Data obtained with UAVs can generate predictive biomass maps with acceptable accuracy levels. The ceptometer leaf area index is a reliable method to estimate forage yield. However, using the canopy height method is not advisable to estimate forage yield, since its correlation is weak.

Keywords: Grassland monitoring, aerial biomass, UAV, ceptometer.

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INTRODUCTION

Grassland ecosystems produce most of the forage needed by livestock and provide essential ecosystem services regarding soil quality, water balance, atmospheric balance, and more. Besides, they have an abundant biodiversity —the basis of any functioning ecosystem (Stumpf *et al.*, 2020). Grassland conditions can be classified according to height, biomass, productivity levels, species composition, and variations of all these factors regarding prior

Image by DJI-Agras at Pixabay

recording stages (Ali *et al.*, 2016). Monitoring the biophysical parameters of grasslands is relevant to determine their development and their relation to the environment; it is also useful for management systems (Acorsi *et al.*, 2019). Estimating parameters such as plant biomass and height is essential to predict yield and to optimize ecosystem management. However, *in situ* measurements can be an arduous and expensive task (Castro *et al.*, 2020).

Accurate, real-time biomass estimates allow producers to meet their management plan goals, leading to better pasture utilization, increased grass growth rates, and enhanced general productivity (Andersson *et al.*, 2017). Advancements in digital agriculture and computer tools, unmanned aerial vehicles, and multispectral cameras make it possible to acquire reliable data, such as vegetation indexes and biophysical parameters (Dos Santos *et al.*, 2020). Although sensors are expensive —preventing a wider use of remote sensing technology— tri-band cameras (RGB) are an inexpensive remote sensing tool for continuous observation (Fu *et al.*, 2021).

Ground-based sensors are yet another option to obtain biomass estimates and offer rapid, automated measurements of spectral reflectance and plant parameter data, such as photosynthetically active radiation (PAR) and leaf area index (LAI). Combined with satellites and other field observation techniques, these sensors are likewise useful to monitor variables —including aerial biomass, agricultural yield, CO_2 uptake, and water stress (Sesnie *et al.*, 2018). Determining biomass availability is essential for an adequate planning, since strategizing involves establishing the load capacity of grazing systems, grass growth, nutritional value, grazing regimes, and loading methods (Batistoti *et al.*, 2019). Therefore, the objective of this study was to evaluate three biomass estimation methods in an arborescent tufted grassland in the state of Chihuahua. The accuracy of the drone method, the ceptometer method, and the canopy height method were compared with the accuracy of quadrant method and the results were then analyzed.

MATERIALS AND METHODS

The study was conducted in an arborescent tufted grassland at the Teseachi ranch, Namiquipa, in October 2020, during the final growth stage of grass. To implement the sampling methods, thirty random points were established, 50 m apart from each other. The first sampling was carried out with an unmanned aerial vehicle (drone). Once the drone flights were completed, a 1 m^2 quadrant was placed in each point and the coordinates were taken. Nine readings were taken with the ceptometer within the quadrant and an average was obtained. Later, the average canopy height within the quadrant was measured. Finally, all the forage was cut at ground level and the quadrant's sampling was packed for laboratory analysis. The sampling made with the quadrant for comparison purposes was the control method.

Unmanned aerial vehicle

Images were captured at an altitude of 100 m, covering approximately 40 ha. We used a professional, quad-propeller DJI Phantom 4 drone with an integrated 4k camera, which has a 94° viewing angle and an f/2.8 aperture lens, with a maximum image size of 4,000×3,000 pixels. The Agisoft Metashape software was used to process the SfM of aerial images.

After the biomass information was obtained, nine sampling points were associated with the coordinates, in order to obtain the digital values of the resulting orthomosaic. The NGBDI vegetation index (Normalized Green-Blue Difference Index) was applied to calculate the average value of all pixels occupying the plot in a 3×3 m moving window. The NGBDI is a normalized ratio of the difference between the green and the blue bands, according to the following formula:

$$NGBDI = (G - B)/(G + B)$$

Ceptometer method

An ACCUPAR LP-80 ceptometer was used to measure fractional PAR (photosynthetically active radiation). This ceptometer has 80 radiation sensors with a waveband of 400 to 700 nanometers (spectrum). Based on a leaf area index (LAI), the ceptometer was used to calculate the available forage at the time. At each sampling site, we conducted nine readings and obtained an average LAI. The ceptometer was calibrated before each sampling. The canopy growth and light interception, as well as the fractional interception, can be determined through this kind of sampling. This method is mostly automated, since it measures the photosynthetically active radiation that strikes the measuring rod. In addition to the automated measurements, data is automatically collected and stored.

Canopy height method

The canopy height within the quadrant was measured by hand, using a ruler with a 0.01 m accuracy. Height was defined as the vertical distance from the ground surface to the average point of the canopy touching the ruler.

Quadrant method

This method is widely used and provides more homogeneous samples for biomass measurements. We established 1 m^2 quadrants (1.25 m×0.8 m). The aerial biomass was cut using a sickle and collected in marked paper bags, labelled with the coordinates. Subsequently, the content of the bags was dried in ovens for 72 hours at 70° C, in order to obtain the dry weight values in grams per square meter and the dry matter values in kilograms per hectare (gr/m^{-2} and kg ha^{-1}) for each site.

Statistical analysis

A simple linear regression model was used to analyze the sampling data, in order to quantify the intensity of linear association between two variables. The R Project free access software, version 4.0.3, was used for this procedure.

RESULTS AND DISCUSSION

Unmanned aerial vehicle

From the images captured by the UAV, an orthomosaic was generated showing the composition of the arborescent tufted grassland area under study in false color (RGB)

(Figure 1). The simple linear regression model —which contrasted the biomass variable with the drone's spectral data— showed a relation to the biomass of the arborescent tufted grassland biomass, with a 0.62 R^2 value ($p<0.01$) (Figure 2). This is consistent with the values reported by Grüner *et al.* (2019) when predicting biomass in a heterogeneous temperate grassland with an SfM approach based on UAV images. They recorded a 0.56 and 0.7 R^2. Lussem *et al.* (2019) estimated biomass in temperate grasslands with high-resolution canopy surface models generated from RGB images and UAV-based vegetation indexes. They determined 0.57 to 0.73 R^2 results. These values concur with those obtained in this study.

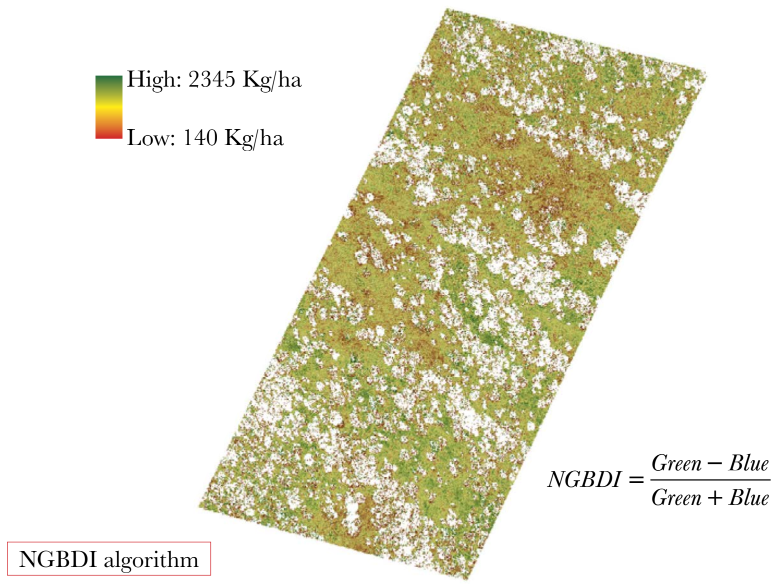

Figure 1. Orthomosaic in RGB (red-green-blue) composition for arborescent tufted grassland.

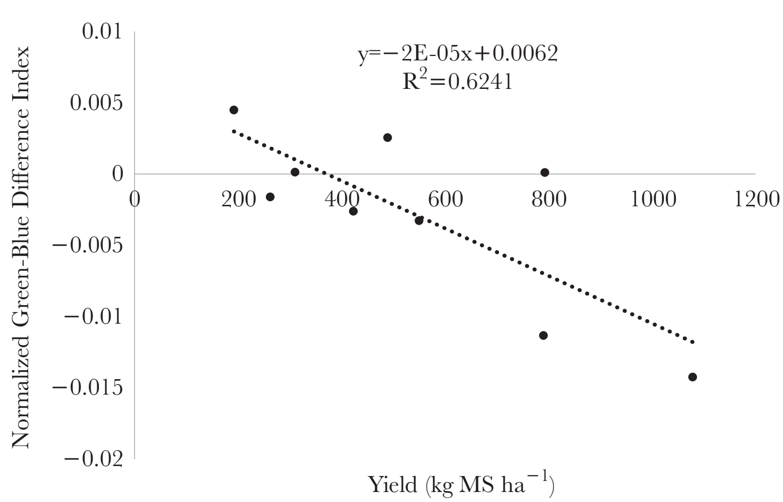

Figure 2. Simple linear regression between drone leaf area index and forage yield in arborescent tufted grassland.

Ceptometer method

The simple linear regression model showed a significant association ($p<0.001$) between leaf area index and forage yield in the samples of arborescent tufted grassland, with a degree of positive association and a 0.55 R^2 (Figure 3). This value is lower than the R^2 reported by Lu *et al.* (2021), who obtained >0.75 values when estimating the spectral and biophysical properties of photosynthetic and non-photosynthetic vegetation in mixed grasslands. For their part, Xu *et al.* (2018) quantified the effects of grazing, the weather, and their interactions in grasslands, and obtained a 0.66 R^2.

Canopy height method

The simple linear regression model showed a significant association ($p<0.001$) between canopy height and forage yield in the samples of arborescent tufted grassland. The model obtained a 0.48 R^2 (Figure 4). This value was similar to the one reported by Grüner *et al.* (2019), who worked on a biomass prediction of heterogeneous temperate grasslands using

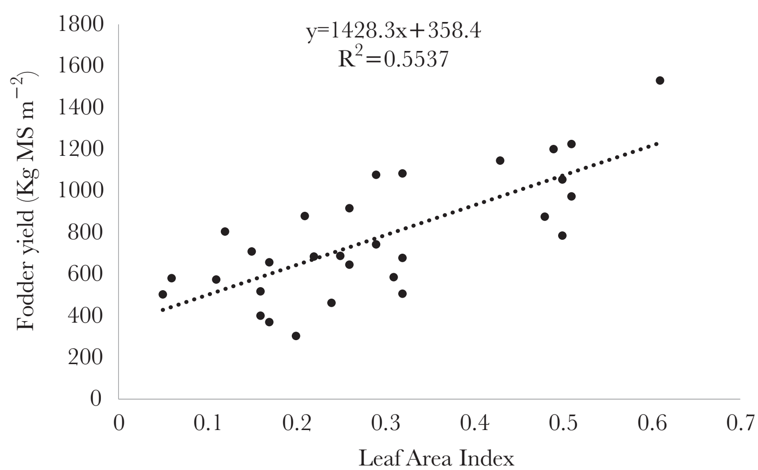

Figure 3. Simple linear regression between the ceptometer leaf area index and forage yield in arborescent tufted grassland.

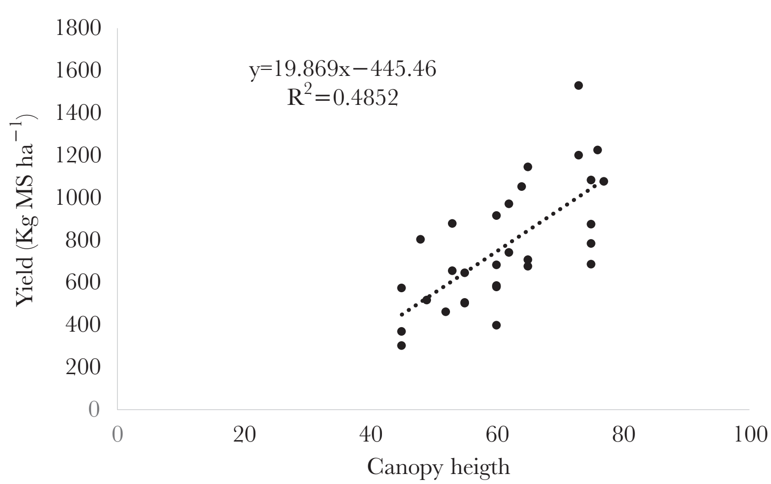

Figure 4. Simple linear regression between canopy height and forage yield in arborescent tufted grassland.

an SfM approach based on UAV imaging, where the linear relation for plant height in pure grass treatments showed a <0.47 R^2. It is important to consider that a correlation is generally low with a <30 absolute value; that the association is moderate when the absolute value ranges from 0.30 to 0.70; and that it is high when it exceeds 0.70.

CONCLUSIONS

UAV data related to field variables of arborescent tufted grasslands in the state of Chihuahua generated through an SfM-based image processing can produce reasonably accurate biomass predictive maps. The leaf area index obtained with a ceptometer can be used to obtain a reliable estimation of the forage yield in arborescent tufted grasslands in the state of Chihuahua, with an acceptable margin of error. This variable is strongly correlated and can be used to estimate biomass production. On the contrary, using the canopy height method to estimate forage yield is unadvisable: it showed a weak correlation in an arborescent tufted grassland in the state of Chihuahua.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Study of the green spaces of the southern area of the city of Chihuahua, Chihuahua, Mexico

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ABSTRACT

Objective: To diagnose the maintenance of green spaces in the southern zone of the city of Chihuahua, Chihuahua, México.

Design/Methodology/Approach: An analytical-synthetic and historical methodology was used to evaluate the state of green spaces, mainly in the southern part of the city. Likewise, bibliographic techniques were used to study the importance of green spaces, their conceptualization, and the challenges that they face.

Results: Providing maintenance and participating in proper management is essential for the improvement of green spaces and for the creation of an enhanced environment that provides better development for these areas in the city of Chihuahua and changes territorial development.

Study Limitations/Implications: Green spaces are part of the basic public amenities of any municipality and therefore the Municipal Council is in charge of these areas; however, the ability to achieve goals is determined by individual and collective productivity or rather the lack thereof.

Findings/Conclusions: Developing good management practices can provide well-kept and adequately maintained green spaces for the inhabitants of Chihuahua.

Keywords: improvement, quality, green spaces, management.

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INTRODUCTION

The purpose of green spaces (particularly parks) is to preserve nature in a highly transformed context (a city). Green spaces are part of the landscape and improve the quality of the urban environment components (Corona, 2001) (Table 1).

The urban revolution that began in the 19th century had an influence on the planning of green spaces in terms of their function and composition The population increase and its resulting pressure gave rise to a feeling of "need for green spaces", involving recreation for citizens and the environmental improvement of the cities themselves. Likewise, the first International Congresses on Hygiene and Urban Planning Problems held in Europe recommended allocating at least 15% of urban areas to parks and gardens and having a forest reserve not less than ten kilometers away from the city (López, 1991). Urban

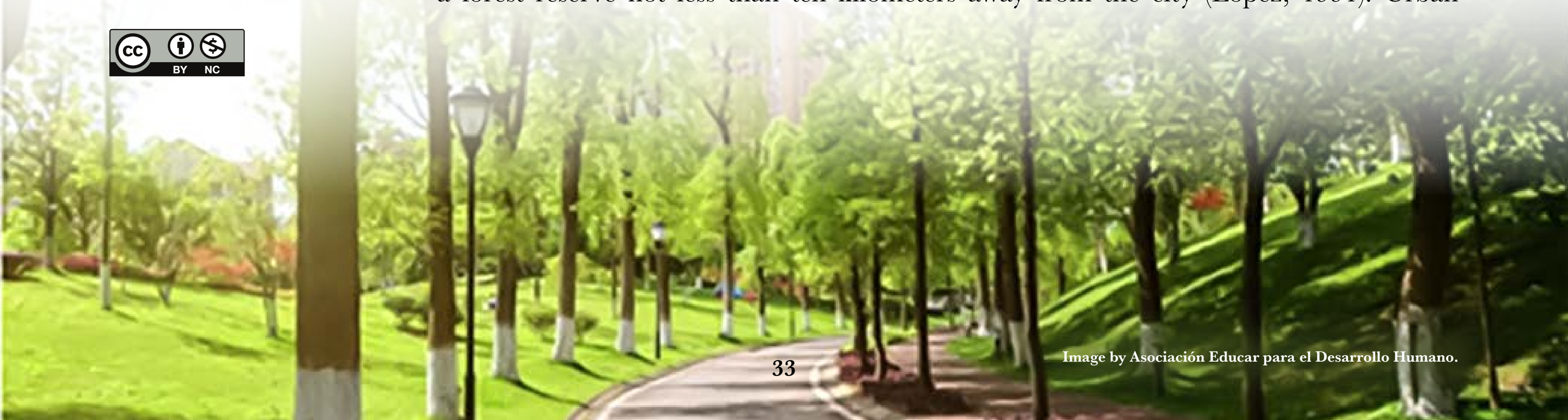


Image by Asociación Educar para el Desarrollo Humano.

Table 1. Purposes of green areas (parks) in an urban environment.

Value	Functions
Ecological	• Aquifer recharge • Control in the emission of particles • Habitat of flora and fauna • Biodiversity • They absorb noise • Microclimate
Architectural Landscape	• Road control visual breakdown • Reduce glare and reflection from the sun • Elements harmonize teas and transition • Improve the physiognomy of the place
Socioeconomic	• Develops sports and cultural activities • Allow environmental education • Provides mental comfort • Pleasant in your moments of rest • Moderate stress offers physical/mental health • Provide employment • Provides material goods • Promotes community coexistence • Increases the price of property

Source: Corona, 2001.

vegetation characterizes and gives its name to green spaces in the city. This allows buildings and people to merge with nature through gardens and parks generating the cityscape: a landscape with an identity given by humans and their culture. Fortunately, many people have become aware of the need for green spaces in the urban environment; these spaces provide a set of essential functions for the well-being and quality of life (Meza and Moncada, 2010). Green spaces also help to regulate urban climate, absorb pollutants, dampen noise, and harvest rainwater, but above all, they create environmental balances in soil, water, and air that are essential for urban environments (Procuraduría Ambiental y del Ordenamiento Territorial, 2003). However, a correct comprehensive analysis that takes into account the territory characteristics and development planning is required to fulfill that purpose (Anchondo and Piñón, 2021). The objective of this article was to diagnose the implementation and maintenance of green spaces in the southern part of the city of Chihuahua, Mexico, in order to determine the perception that the population and community have about them, as well as to establish their concerns, needs, conflicts, and the improvements that they deem necessary.

MATERIALS AND METHODS

To know and establish the bases for a diagnosis of green spaces with poor conditions and subsequently involve the population of the city of Chihuahua, a qualitative survey was carried out using questionnaires in the Rosario, Gustavo Díaz Ordaz, Ampliación Díaz Ordaz, and Dale-UP neighborhoods in the southern area of the said city. This questionnaire was administered to a total of 100 people and the Statistical Package for Social Sciences version program was used for analyzing the resulting data.

RESULTS AND DISCUSSIONS

The results obtained are shown below and each question is subject to an individual analysis to determine the trend or magnitude.

Figure 1 shows that 55% of the participants of the survey does not frequent parks or green spaces, even though the population of the city of Chihuahua should visit these spaces with greater frequency.

Regarding the awareness of the population about the number of urban parks that exist in the southern area of the city, Figure 2 shows that most of the population (95%) surveyed does not know how many urban parks are located in that area.

Figure 3 shows that 60% of the urban population considers that green spaces are very important; therefore, we can conclude that the population is aware of the importance of these green spaces for society. It is also worth mentioning that just a small part of the population thinks that these spaces are not of the highest importance: only 10% of the surveyed population answered, "very little" and 5% replied "not at all".

Figure 4 shows that 80% of respondents mentioned that, according to their experience, urban parks are currently in very poor condition, which points towards a great area of opportunity within the city.

Regarding perception of cleanliness, maintenance, and facilities —as well as area lacking in the urban parks—, Figure 5 shows that 60% of the population surveyed answered

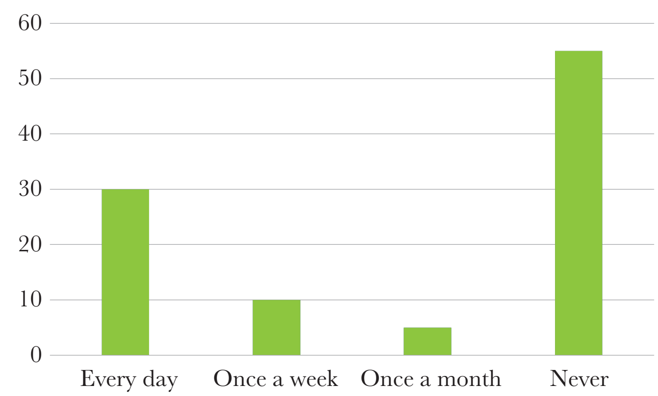


Figure 1. Frequency of visits to parks or green spaces. Source: table developed by the authors.

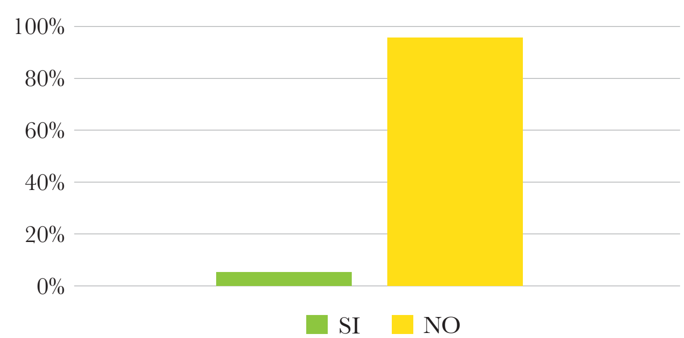


Figure 2. Number of urban parks in the southern zone of the city of Chihuahua. Source: table developed by the authors.

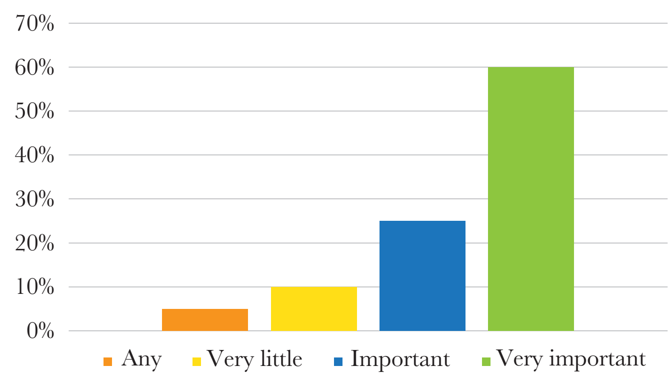


Figure 3. Importance of green spaces within the city. Source: table developed by the authors.

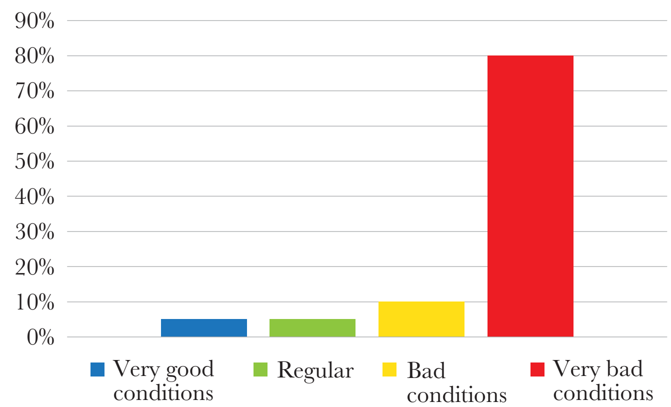


Figure 4. Conditions of the urban parks. Source: table developed by the authors.

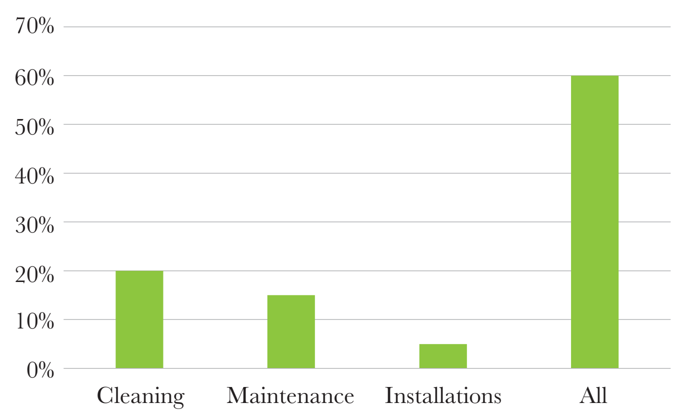


Figure 5. Aspect lacking in urban parks. Source: table developed by the authors.

"all areas", 20% "cleanliness", 15% "maintenance" and 5% "facilities". According to the said figure, people clearly feel that "all areas" of urban parks (cleanliness, maintenance, and facilities) require attention. Keeping urban parks clean is very important: the lack of maintenance and facilities results in dirty or damaged areas that will not be attractive to the inhabitants, who therefore will not have any interest in them whatsoever.

As can be seen in Figure 6, 70% of the population considered that the quality of most urban parks is low, while 20% considered that they have a medium level and only 10% considered that their quality is high. It should be highlighted that the population considered that high-quality green spaces include sports facilities and/or water bodies (dams).

Figure 7 shows that 80% of the respondents consider that green spaces are unsafe. It should be mentioned that the majority of the population voiced their concerns about the very poor condition of public lighting in green spaces and the lack of surveillance by the authorities that make them unsafe spaces. Only 5% of the population considered green spaces to be very safe; however, those respondents live in areas with greater security surveillance (such as residential developments).

In relation to self-sustaining parks, Figure 8 shows that 92% of the population thinks that more self-sustaining areas and parks are required, while only 8% thought the opposite. It should be noted that the latter negative responses belong to people who do not use this kind of facilities (*e.g.*, urban parks, dams, and sports facilities).

Based on the results obtained, Figure 9 shows the reasons why the population does not visit the different green spaces: 30% of the inhabitants mention poor conditions, while another 30% blames damaged facilities. Another noteworthy reasons maybe that even

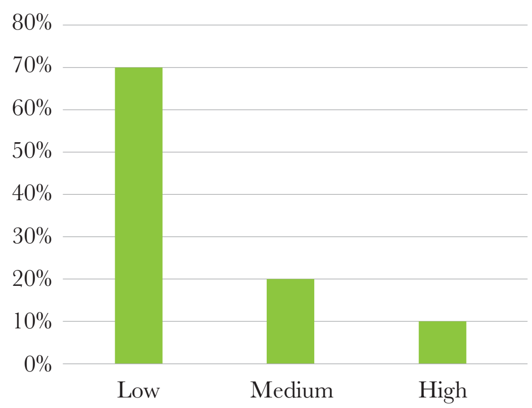


Figure 6. Quality of urban parks. Source: table developed by the authors.

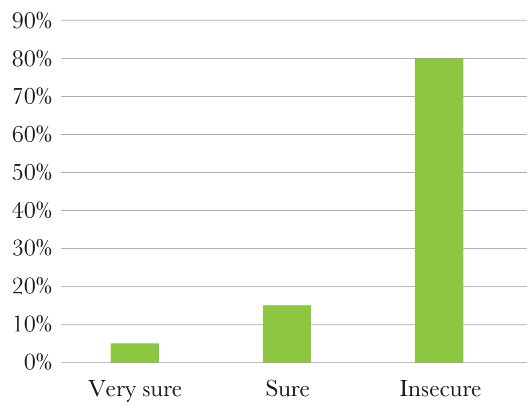


Figure 7. Safety in green spaces. Source: table developed by the authors.

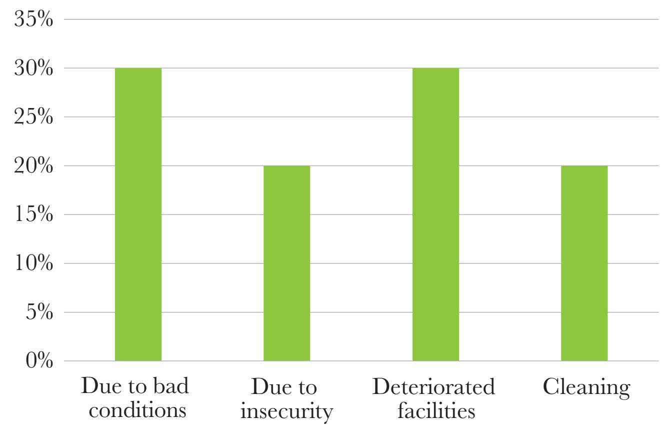


Figure 8. Reasons why the population does not visit green spaces. Source: table developed by the authors.

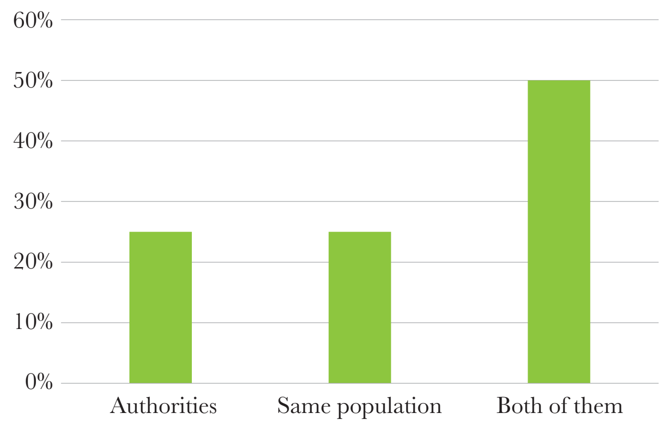


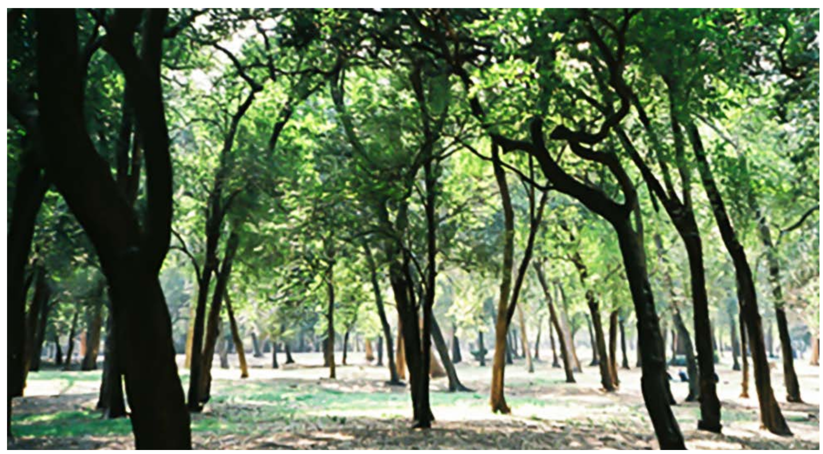
Figure 9. Responsibility for the carelessness of green spaces. Source: table developed by the authors.

parks in good condition can have very damaged and old facilities. Another major issue (mentioned by 20% of the respondents) is insecurity. Regarding cleanliness, 20% of the population considered that this is another very important reason why the inhabitants do not visit these areas.

As can be seen in Figure 10, 50% of the population considered that both the authorities and the population itself are responsible for the carelessness and damage of these green

Figure 10. Park located in Ampliación Díaz Ordaz. Source: photo retrieved by the authors from Google Earth.

Figure 11. Park located in Gustavo Díaz Ordaz. Source: photo retrieved by the authors from Google Earth.



Picture of Bosque de Chapultepec. Source: Travel Report.

spaces, while 25% believed that its solely the responsibility of the authorities who oversee the maintenance of these green spaces. The remaining 25% believed that the population itself frequently causes the deterioration of these areas.

CONCLUSIONS

This problem has been caused by the neglect of the green spaces of the city of Chihuahua. Currently, they are frequently seen in a significant level of abandonment, resulting in the deterioration of these facilities and the lack of interest among the population. The lack of services (*e.g.*, public lighting) has caused the population to refrain from visiting these areas, out of fear or as a result of the neglect or lack of cleanliness of these spaces. Public spaces must be rescued and created to make the city a place of social integration that offers better habitability, comfort, and equity.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Geographic distribution of the production of certified corn (*Zea mays* L.) seed

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ABSTRACT

Objective: To analyze the effects of a greater geographic dispersion of municipalities that produce and consume certified corn seed in the State of Mexico.

Design/Methodology/Approach: A solution for the spatial equilibrium model applied to the certified seed market in the State of Mexico was reached in 2019.

Results: The results indicate that a greater geographical dispersion of producing and consuming municipalities would reduce production costs by 0.8% and increase income, distribution costs, and profits by 0.1, 3.0, and 1.3%, respectively, as compared to the data observed in the studied year.

Study Limitations/Implications: The positive effects observed in this study suggest that a greater geographical dispersion would benefit regions whose municipalities have a high potential to produce certified seed.

Conclusions: In addition to having a moderate effect on profits, the greater geographic dispersion would benefit corn producers, who would have a greater access to certified seed. As a result, both the seed use rate in the region and the yield level per hectare would increase.

Keywords: Corn, Certified seed, Geographic concentration, Earnings, Spatial equilibrium model.

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INTRODUCTION

The State of Mexico uses around 54% of its agricultural area for the cultivation of corn (INEGI, 2016), which accounts for 44% of the production value in the state's agricultural sector (SIAP, 2020).

During the 2010-2019 period, corn production experienced an average annual growth of 3%. Part of this growth resulted from a 2% yield increase, which shows a rise in productivity per surface unit (SIAP, 2020). Among other factors, this increase in productivity is the result of the intensified adoption of technological packages (Rodríguez and Donnet, 2015). Technological use data indicate that, in 2014, 29% of the state's cornfields used some type of improved seed, while in 2020 the use rate of improved corn seed was close to 40% (SIAP, 2017). These figures —which are similar to those estimated by Donnet *et al.* (2020)—

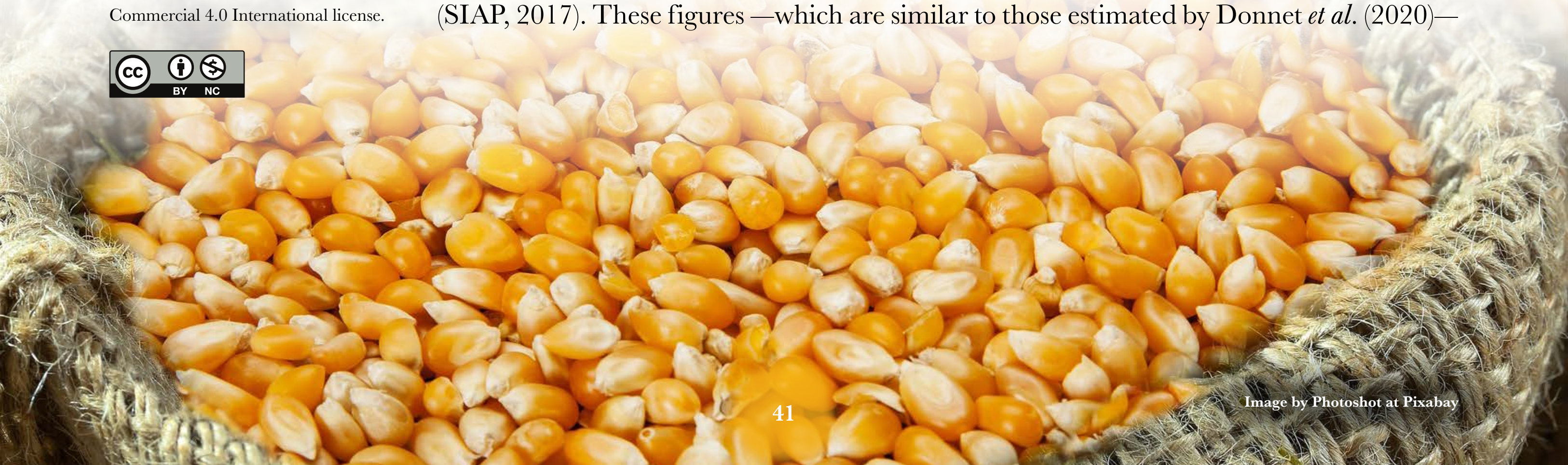

Image by Photoshot at Pixabay

indicate a slow (2%) but constant increase in the use of improved seeds in the state and show that most cornfields do not use this type of seeds.

The low use rate of improved seed, along with a slow adoption process, is caused by, among other things, the prevalence of small farms. Statistics from the 2017 agriculture census indicate that 60% of the land used for open sky agriculture in the State of Mexico is cultivated by small farmers in plots of 0-5 ha (INEGI, 2016); these farmers have financial difficulties. INEGI (2017) reports that 81.7% of the producers who received a loan used it to buy improved supplies.

For small- and mid-scale producers, the final price of the seed (determined by the type of hybrid), as well as its availability and accessibility, is decisive when deciding whether or not to use improved seeds (Sierra-Macías *et al.*, 2016).

Certified seed is produced in areas of the State of Mexico where grain corn is produced, 60% of which are concentrated in the Rural Development District (RDD) of Atlacomulco, 20% in Toluca, and the rest in Texcoco (SNICS, 2020).

Unlike Sinaloa, Jalisco, or Guanajuato, the companies that produce certified seed in the State of Mexico are small companies whose production is below 50 tons (MacRobert, 2014). Virgen-Vargas *et al.* (2016) identified 35 companies in the high valleys. Eight of those companies produced certified seed in 2019 (SNICS, 2020): 263 tons and an average yield of 2.79 t ha^{-1} during the 2019 spring-summer cycle. These companies produced certified seeds of 11 hybrids, three Open Pollinated Varieties (OPV), and two Synthetic Varieties (SV). Of all the seeds produced in the state, 54% comprise three varieties: H-50, CP-HS2, and H-52. Data of the cultivated area and the use of seed per hectare recommended by INIFAP (2017) indicate that the previous production represents 6.6% of the improved seed (SIAP, 2017) and 2% of all the grain corn seed used in the state.

The concentration of production in three RDDs and its low volume have restricted availability and accessibility for other municipalities that produce corn and have a high use rate of improved seed. This is the case of the District of Zumpango, with a use rate of 78% (SIAP, 2017), which makes it an important area for the production of certified seed.

A difficult access to certified seeds forces producers to acquire improved seeds that do not have an adequate certification or even informal seeds (CEDRSSA, 2015). Likewise, the higher prices resulting from the transportation costs (from the producing to the consuming areas) discourages them altogether from acquiring this kind of seeds. Distribution costs can reach 30% of the overall cost of a sack of seed and it might take as long as 30 days for the orders to be delivered (personal communication, Customer Service and Sales Office. Empresa La Semillera, August 2020).

A spatial redistribution of producing areas would improve the income of seed production companies, as well as the access of consumers to seed. They may even obtain them at better prices, as a result of a reduction in distribution costs in the producing and consuming areas.

Therefore, the aim of this study is to propose a spatial redistribution of certified seed production in the State of Mexico. This redistribution would accomplish the following objectives: a) to improve the certified seed producers' profits; and b) to favor a better access to certified seeds for small- and mid-scale producers who use improved supplies.

MATERIALS AND METHODS

In order to achieve the objectives, a spatial equilibrium model was used to develop a model of the spatial dispersion of certified seed production and consumption. The model is based on Takayama and Judge (1971); the solution seeks to replicate the current operation of the certified corn seed market in the State of Mexico. The scenarios developed consider redistributions of production and consumption that differ from the distribution observed in the base year.

The target function maximizes the net profit as an indicator to measure competitiveness, which equals the income resulting from certified seed sales, minus the costs of production, distribution, and shipment of the certified seed to nearby regions outside the state. The surplus production of the certified seed in producing areas is transported to consuming areas. The model considers the irrigation regime for certified seed production and both water regimes in the case of consumption.

Supposing that $i(i=1,2...I)$ represents the certified corn seed producing regions; $j(j=1,2..J)$ the seed consuming regions; $n(n=1,...N)$ the seed export points (shipment to regions outside the state); and $t(t=1,...T)$ the production cycles, the model could be formulated as follows:

$$\begin{aligned} Max\ Z = & \sum_{t=1}^{T}\sum_{j=1}^{J}\left[p_{jt}y_{jt}\right]+\sum_{t=1}^{T}\sum_{n=1}^{N}p_{nt}y_{nt}-\sum_{t=1}^{T}\sum_{i=1}^{I}cp_{it}x_{it} \\ & -\sum_{t=1}^{T}\sum_{i=1}^{I}\sum_{j=1}^{J}cp_{ijt}x_{ijt}-\sum\nolimits_{t=1}^{T}\sum\nolimits_{i=1}^{I}\sum\nolimits_{n=1}^{N}ct_{int}x_{int} \end{aligned} \quad (1)$$

Where p_{jt} is the price for the seed consumer in j for period t; y_{jt} is the consumption of certified seed in j; cp_{it} is the production cost in i; x_{it} is the amount produced in i; p_{nt} is the price at the export point n; x_{nt}-is the amount exported through n; ct_{ijt} are the transportation costs from i to j; x_{ijt} are the seed shipments from i to j; ct_{int}-are the transportation costs from i to n; x_{int}-in- are the seed shipments from i to n.

The target function is subject to the following restrictions:

$$\sum\nolimits_{i=1}^{I}x_{ijt}\geq y_{jt} \quad (2)$$

$$x_{it}\geq\sum\nolimits_{j=1}^{J}x_{ijt}+\sum\nolimits_{n=1}^{N}x_{int} \quad (3)$$

$$\sum\nolimits_{n=1}^{I}x_{int}\geq x_{nt} \quad (4)$$

$$x_{it},y_{jt},...x_{int}\geq 0 \quad (5)$$

Equation 1 maximizes the net profit, which equals the income resulting from seed sales, minus the costs of production and transportation. Restriction 2 determines how the corn seed demand is met in each consuming area. Restriction 3 indicates how the seed production is distributed. Equation 4 establishes that seed shipments from producing regions in the State of Mexico must surpass seed consumption in regions outside the state. Equation 5 establishes the non-negativity conditions.

The model's solution considers eight producing municipalities, 15 consuming municipalities, and two municipalities adjacent to the State of Mexico. Figure 1 highlights the established producing and consuming municipalities, as well as the adjoining states.

To analyze the benefits of redistributing the certified seed producing areas, first the solution for the model in a base situation was obtained. Subsequently four scenarios were developed. Scenario 1 considered the eight producing municipalities of the base scenario, but increasing the number of consuming municipalities from 17 to 22 (base+potential), and limiting the supply of the production to municipalities within the state. Scenario 2 considered the same municipalities as scenario 1, plus two consumer municipalities outside the state, for a total of 24 municipalities. Scenarios 3 and 4 considered ten producing municipalities (base+potential), while the consuming municipalities remained the same as in scenarios 1 and 2.

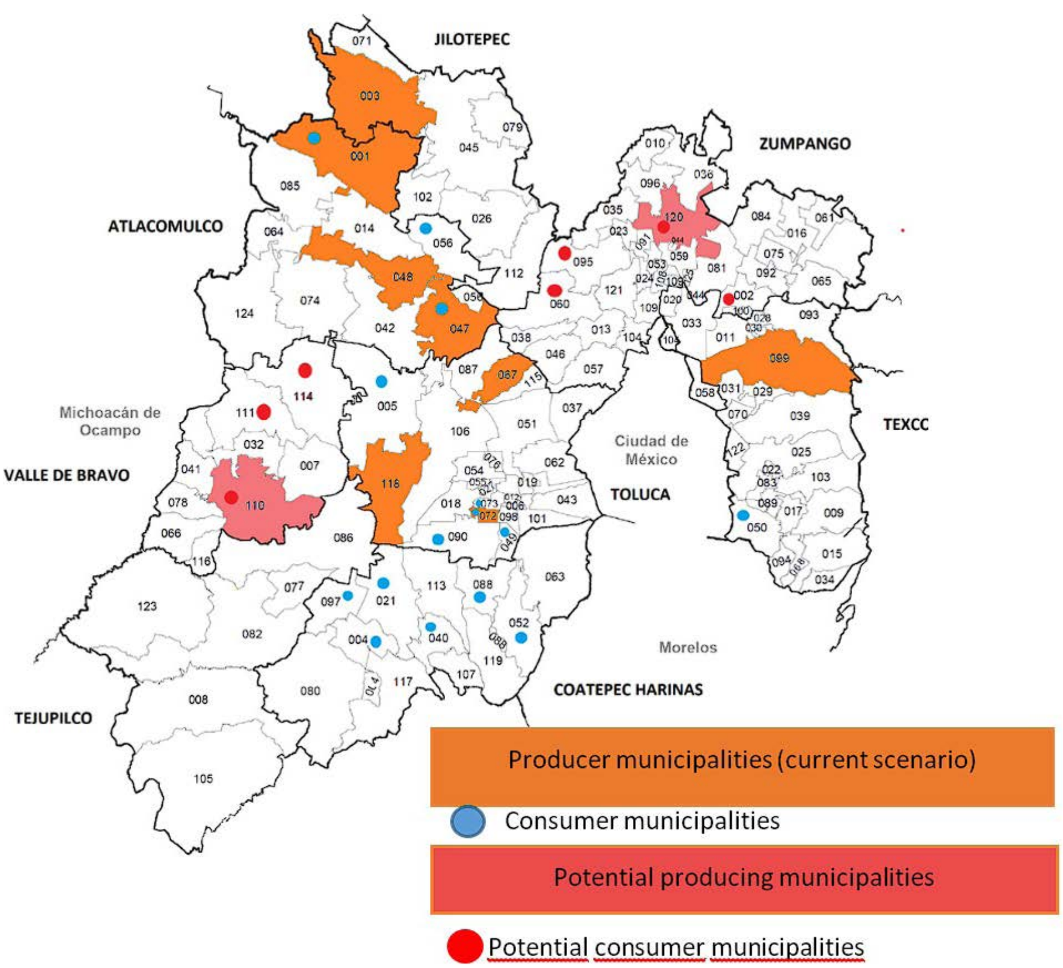

Figure 1. Spatial distribution of current and potential producing municipalities.

The consuming areas of the base and potential scenarios are those that demand certified seed according to the commercialization of corn grain, the area used to grow corn in each municipality, and the yields that suggest the use of agricultural technology (*e.g.*, improved seed, either prepared or certified). The scenario's consuming areas were established based on the following criteria: a) a cultivated area larger than 1,000 ha; b) a yield higher than 4 tons (in average); and 3) a use rate of improved seed higher than 40%.

By applying the aforementioned criteria, were identified 15 consuming municipalities and detected focalized potential consuming areas outside the zones where certified seed production and consumption are currently carried out; however, these areas were determined based on more flexible restrictions. They are located in the RDDs of Zumpango —with a surface of over 700 ha, a yield of 2.5 t ha^{-1}, and a use rate of improved seed higher than 40%— and Valle de Bravo —with a surface of over 700 ha, a yield of over 3.7 t ha^{-1}, and a use rate higher than 25%. The distribution of consumption within each municipality was estimated based on García and Ramírez (2012).

The data were taken from the several sources. Production by water regime and production cycle data were found in Córdova-Téllez *et al.* (2019), SNICS (2020), and SIAP (2020). Seed shipments from the State of Mexico to other states and certified seed production costs were taken from a personal communication from Mancera-Rico (2020), professor-researcher in postharvest seed handling at the Universidad Autónoma Agraria Antonio Narro. The price of the different hybrids was provided by SNICS (2017) and COLPOS (2020). Distribution costs from producing to consuming areas were estimated based on distances and tolls (RCO, 2020). Operation costs in Mexican pesos per vehicle-kilometer for 2019 were found in Arroyo-Osorno (2020), while loading and unloading costs were taken from Transportes SIGFRA (2019). Costs were calculated considering a general quote based on the loading and unloading cost divided by the transported volume. Potential producing areas were determined based on information from SIAP (2017) and the preference for certified seed per municipality was established using data from SIAP (2017; 2020).

RESULTS AND DISCUSSION

Figure 1 shows the current and the potential spatial distribution of producing and consuming municipalities. The current geographic distribution is characterized by a high concentration of municipalities where improved seed is produced and consumed in the Districts of Atlacomulco and Toluca. Meanwhile, the Districts of Zumpango, Valle de Bravo, Tejupilco, and Coatepec Harinas do not have municipalities that produce certified seed.

Table 1 presents some economic indicators related to the production and distribution of improved seed in the base model. Income, production costs, and distribution costs amounted to 15,359, 4,520, and 876 million pesos, respectively, which resulted in a profit of 9,865 million pesos.

Scenario 1 considers the same producing municipalities as the base scenario, but the number of consuming municipalities increases from 15 to 22 (17 municipalities that currently consume certified seed plus five potential consumers). In this scenario, the production of improved seed remains constant and, therefore, production costs do not change; however, more consuming municipalities results in a 12.6% increase in distribution costs. Higher costs, together with a lower income, reduced the profits by 57,000 pesos: a drop of 0.6% in relation to the profits observed in the base model.

Scenario 2 considers the same producing and consuming municipalities as scenario 1, but takes into account 50% more shipments outside the state than the base scenario. This involves sending part of the production outside the State of Mexico, while the rest is distributed among the potential consuming municipalities. The results of scenario 2 show an 0.2% income increase regarding the base scenario. Production costs remain unchanged, while distribution costs increase by 3.0% in relation to the base model. The increase in income is canceled by the increase in distribution costs, resulting in the same profits as in the base model.

The income in scenario 3 drops by 43,000 pesos, which entails a decrease of 0.3% in relation to the base model. Production costs decrease by 0.8% as a result of the type of hybrid suggested in the new municipalities, based on the weather conditions of the proposed areas. Given their location, distribution costs increase by 9.2%, which means that circulating the seed among producing and consuming municipalities within the state is more expensive than sending the seed to municipalities outside the state.

Table 1. Income, costs, and profits of certified seed production and distribution in the State of Mexico, 2019 (thousands MXN).

Scenario	**Municipalities**		**TUSM %**	**Yield t ha^{-1}**	**Income**	**Costs**		**Profit**
	producers	**consumers**				**production**	**distribution**	
					thousands MXN			
Base model	8	17	greater than 40	greater than 4	15,359	4,522	877	9,865
Scenarios								
1	8	22	25 y 40		15,316	4,522	987	9,808
2	8	24	25 y 40		15,382	4,522	903	9,868
3	10	22	25 y 40	between 2.5 and 4	15,316	4,484	957	9,875
4	10	24	25 y 40	between 2.5 and 4	15,382	4,484	903	9,995
Change with respect to the baseline situation								
1	0	5	-	-	−43	0	110	−57
2	0	7	-	-	23	0	26	3
3	2	5	-	-	−43	−38	80	10
4	2	7	-	-	23	−38	26	130
Change in % with respect to the baseline situation								
1	-	-	-	-	−0.3	0.0	12.5	−0.6
2	-	-	-	-	0.1	0.0	3.0	0.0
3	-	-	-	-	−0.3	−0.8	9.1	0.1
4	-	-	-	-	0.1	−0.8	3.0	1.3

Source: Developed by the authors based on the results for the base model and the four scenarios.

Income in scenario 4 increases by 0.2% regarding the base model. Production costs drop by 0.8%, while distribution costs increase by 0.3%. In this scenario, profits were 1.3% higher than in the base model.

Profits do not change significantly from one scenario to the other. This was as expected, since the production of certified seed remains constant; however, we must point out that if certified seed producers took the initiative to produce in these areas, their profits would remain the same. Nevertheless, both accessibility for producers and availability of the certified seed would increase.

These results indicate that changes in the distribution logistics of production and supply (in this case, certified seed) frequently have positive and negative effects on variables such as income, production and distribution costs, and profits for producers. García-Salazar and Skaggs (2015) show similar effects regarding distribution costs after yellow corn replaced white corn in a given area, as a consequence of a change in consumption. When roads or transport routes are in poor conditions or the areas are difficult to access, distribution costs are frequently higher, potentially increasing the time and cost needed to reach the consuming areas (Ayllon-Benítez *et al.*, 2015). This situation affects producers with less accessibility and lower consumption levels. Preference is given to nearby areas (in this case, in adjacent states), thus hindering the growth of consuming areas within the state. Similarly, seed costs for grain producers can vary significantly if distribution costs are reduced and accessibility is improved.

CONCLUSIONS

Currently, the geographic distribution of improved seed production and consumption is notably concentrated in the RDDs of Atlacomulco, and Toluca, and to a lesser extent in Texcoco. Some municipalities have the potential to become certified seed producers and consumers, which indicates that dispersing the municipalities that already produce and consume certified seed is possible.

The solution of a spatial equilibrium model applied to the certified seed market in the State of Mexico indicates that a better spatial distribution of production and consumption —by means of a geographical dispersion of municipalities with the potential to produce and consume improved seed— would improve distribution costs, but would not have any effects on producers' profits. If the certified seed were sold in regions outside the State of Mexico, the seed-producing companies would see moderate increase in their profits.

The geographic dispersion of the certified seed production in the different municipalities would entail an improvement in the access of producers to the improved seed, which would encourage its use and dissemination, and would therefore lead to an increase in the use rate of certified seed. Bringing the producing and consuming areas closer would also improve prices, encouraging producers to change from low potential seeds to seeds with a higher production potential.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Profitability of Corn (*Zea mays* L.) Grown in *Milpa* Production Systems in Oaxaca, Puebla, and Veracruz, Mexico

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ABSTRACT

Objective: To compare the profitability of corn cultivation under the *milpa* production system in Oaxaca, Puebla, and Veracruz.

Methodology: Ninety-one producers, from Villa de Zaachila, Oaxaca, Chalchicomula de Sesma, Puebla, and Tequila, Veracruz, Mexico, were interviewed about corn production in *milpa* production systems. Average yield per hectare, costs, selling price, and total income were compared and the Cost Benefit Ratio (CBR) of the *milpas* of each municipality was analyzed.

Results: In Villa de Zaachila, corn production is carried out in a modern agriculture system with a \$13,650 ha^{-1} total production costs, a 2,257 kg ha^{-1} yield, a \$23,429.27 ha^{-1} total income, and a 1.72 CBR. The producers of Chalchicomula de Sesma use a traditional agriculture system and the production is sold to intermediaries, with a \$12,380 ha^{-1} total cost, a 2,456 kg ha^{-1} yield, a \$12,280.00 ha^{-1} total income, and a 0.99 CBR. In Tequila, a subsistence agriculture is used, with a \$7,350 ha^{-1} total production cost, a 964 kg ha^{-1} yield, a \$6,748.00 ha^{-1} total income, and a 0.92 CBR.

Study Limitations: The data were taken from an exploratory sample, limited in time and space. Non-sampled versions or productive practices could have been omitted, when technical-economic specificities are different from those shown in this study.

Conclusions: The contrasting modifications in the structure, function, and logic of the *milpa* (2020-2021 cycle) resulted in a profitable agroecosystem in Villa de Zaachila; however, Chalchicomula de Sesma and Tequila had a non-profitable cycle.

Keywords: Traditional agriculture, Rural development, Agricultural Economics, Farming.

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INTRODUCTION

In Mexico, corn (*Zea mays* L.) is fundamental to the culinary and cultural traditions of the rural society (Leyva *et al.*, 2020; Novotny *et al.*, 2021). The acceptance and reproduction of this crop is based on the diversification of its use and the adaptation of the 68 races to their

Image by Andrew Martin at Pixabay

uses and the different weather conditions (Caballero, Córdova, and López, 2019). Only 22% of the arable land in Mexico can be considered as high-quality land with irrigation, while 78% of the arable land is used for rainfed agriculture. In the latter area, corn crops are grown under *milpa* production systems. Corn is the main product of traditional agriculture (Turrent *et al.*, 2014; Rodríguez *et al.*, 2020; López *et al.*, 2021).

The *milpa* is a complex system with synergies between its plants and weeds, such as tolerance, protection, nutrient fixation, and pest management, among others (Caballero and Cortés, 2001; Eyzaguirre and Linares, 2004). In practice, these agroecosystems are dynamic and adaptable to such an extent that they have currently diversified from polycultures to monocultures to satisfy the soil, market, or destination of the production requirements (Guzmán, 2016; Sosa and González, 2021).

In this scenario, analyzing the different productive aspects of the said agroecosystem's regional adaptations is fundamental. This analysis compares those regional adaptations with the adaptations, improvement points, and agronomic optimization required to increase the agricultural yields, according to the requirements of the farmers and their ethno-biological uses.

Consequently, the objective of this study was to compare the yield of corn crops grown under *milpa* production systems, in three different scenarios. The productive versions of this agroecosystem were compared based on the data from the Valles Centrales in Oaxaca, Valle de Serdán in Puebla, and Zongolica, in Veracruz. The hypothesis was that —according to the dynamism of the *milpa* as an adapted agricultural practice-contrasting modifications would be found in the structure, function, and logic of the most profitable production model for each context.

MATERIALS AND METHODS

This research was developed within the framework of the Proyecto Nacional de Investigación e Incidencia "Estudio agroecológico del sistema *milpa* en Veracruz, Oaxaca y Puebla" organized by the Consejo Nacional de Ciencia y Tecnología (CONACyT). As contrasting measure, the study area included municipalities with different degrees of technology innovation in the *milpa* and contradictory environmental conditions, as well as recent adaptations of the agroecosystem (INEGI, 1995; INEGI, 1997; INEGI, 2009; SEDESOL, 2012; SEGOB Veracruz, 2019). The municipalities of Villa de Zaachila, Chalchicomula de Sesma, and Tequila were chosen based on these criteria (Table 1).

An exploratory interview tool was designed. This tool included specific data of the *milpa* production system, such as: a) size and ownership of the plot; b) sowing and crop association data; c) tools and inclusion of technology; d) pests and diseases; e) harvest, treatment, and destination of the production.

From december 2020 to april 2021, producers linked to *milpa* production systems were interviewed about their productive characteristics. The interviewees were divided as follows: 25 from the Villa de Zaachila municipality, in the Valles Centrales region, Oaxaca; 25 from the Chalchicomula de Sesma municipality, Puebla, in Región III Valle de Serdán (Ciudad Serdán); and 41 from the Tequila municipality in the Las Montañas

Table 1. Comparison of the study areas: Villa de Zaachila, Chalchicomula de Sesma, and Tequila.

	Villa de Zaachila, Valles Centrales, Oaxaca	Chalchicomula de Sesma, Valle de Serdán, Puebla	Tequila, Las Montañas, Veracruz
Physiographic province	Southern Sierra Madre, sierras, and valleys of Oaxaca sub province	Neovolcanix Axis, lakes and volcanos of Anáhuac sub province	Southern Sierra Madre, Eastern sierras sub province
Weather	BS1h'(h)w(w)i'gw"	Cb(W0) i'g	(A)C(W2) ig
Soils	Vertosols, regosols, and leptosols	Regosols	Luvisols
Type of agriculture	Modern	Traditional	Subsistence

region (Zongolica), Veracruz. The participants were chosen based on the following factors: a) being a member of a farmer family, b) living in the chosen communities, and c) growing native corn crops in monoculture or association.

The data from the interviews were analyzed as a whole, in order to create a hypothetic plot per each group of producers. The values of costs and benefits were analyzed in Mexican pesos (MXN). Each analyzed municipality produced 1 ha of corn during the 2020-2021 period. To calculate the yield, the following basic economic variables were determined:

a) Total costs (TC). The productive costs were estimated according to the work stage: 1) land preparation, 2) sowing, 3) fertilization, 4) pest and disease control, 5) weed control, 6) support labors, and 7) harvest. The following formula was used to calculate the TC calculus:

$$TC = FC + VC$$

b) Fixed costs (FC). Constant costs that do not change in relation with the production volume.
c) Variable costs (VC). Costs that are modified in relation with production volume.
d) Total income (TI). Income that could be obtained when the whole harvest is sold (Q) at the average price of the region (P).

$$TI = P * Q$$

e) Sales revenue (SR). The TI formula was considered, but it was adjusted to the mean proportion of the harvest that is sold in each study area.
f) Self-consumption opportunity cost (OC). The SR and Self-Consumption Revenue were established, and the Significant Difference Method was used to determine the Opportunity Cost.

$$Opportunity\ Cost = Total\ income - Self - Consumption\ Revenue$$

g) Cost-Benefit Ratio (CBR). This value is obtained dividing the Net Present Benefit Values (NPBV) by the Total Cost Present Value (TCPV). A positive yield factor (higher than 1) result in a profitable CBR.

$$CBR = \frac{NPBV}{TCPV}$$

RESULTS AND DISCUSSION

Villa de Zaachila, Oaxaca

According to the survey conducted in this research, the total average size of the production units in Villa de Zaachila is 2.16 ha and 65% of the interviewees owned their production unit. Fourteen different crops or soil uses were mentioned and corn production accounts for 68.1% of the arable land. For the purposes of this research, corn production in Villa de Zaachila is considered as a modern agricultural system.

The farmers in the region that have irrigation and consequently can produce two cycles per year amount to 58.3% of the local producers. The crops sown were improved corn (53.8%) and bolita corn, associated to the following crops: crookneck squash (*Cucurbita moschata*), acorn squash (*Cucurbita pepo*), silver-seed gourd (*Cucubita argyrosperma*), and bean (*Phaseolus vulgaris*). Eighty-one percent of the interviewees use a tractor; 80.7% use chemical fertilization methods; 57.7% use chemical products to control pests and diseases; and 38.46% use mainly yokes to control the weed. Table 2 shows that the total cost of corn production for the 2020-2021 cycle in Villa de Zaachila amounted to \$13,650.00 ha^{-1}.

The average harvest (Q) reached 2,257 kg ha^{-1} and the production is mainly destined for sales, forage, and family consumption. The selling price (P) amounted to \$35.00 per bushel (approximately 3.5 kg), which means that the price was \$10.00 kg^{-1}. The TI was \$23,429.27 ha^{-1}; however, only a fraction of the harvest is sold. In average, this fraction amounted to 1,896 kg ha^{-1}, which means that the SR of the 2020-2021 cycle was just \$18,963.64 ha^{-1}. Table 3 shows that the OC reached \$4,465.63 ha^{-1}.

Table 2. Total cost of the corn production in Villa de Zaachila, for the 2020-2021 cycle.

Activity	Fixed Costs	Variable Costs	Cost per Activity
Land preparation (\$ ha^{-1})	1,050.00	2,700.00	3,750.00
Sowing (\$ ha^{-1})	1,700.00	2,200.00	3,900.00
Fertilization (\$ ha^{-1})	0.00	1,420.00	1,420.00
Pest and disease control (\$ ha^{-1})	400.00	380.00	780.00
Weed control (\$ ha^{-1})	800.00	0.00	800.00
Support labor (\$ ha^{-1})	0.00	0.00	0.00
Harvest (\$ ha^{-1})	3,000.00	0.00	3,000.00
TOTAL (\$ ha^{-1})	6,950.00	6,700.00	13,650.00

Table 3. Corn production Income in Villa de Zaachila for the 2020-2021 cycle.

Harvested Corn (Q)	Selling Price (P)	Total Income (TI)	Average Amount of Sold Corn	Sale Revenue (SR)	Self-Consumption Opportunity Cost (OC)
2,343 kg ha^{-1}	10.00 \$ kg^{-1}	23,429.27 \$ ha^{-1}	1,896 kg ha^{-1}	18,963.64 \$ ha^{-1}	4,465.63 \$ ha^{-1}

Chalchicomula de Sesma, Puebla

In the communities of San Francisco Cuautlancingo and Santa Cruz Veladero, Chalchicomula de Sesma municipality, Región Valle de Serdán, the total average size of the production units is 4.98 ha and 78% of the interviewees own their production units. Nine different crops were mentioned; corn is sown in 52.37% of the arable land. For the purposes of this research, corn production in Chalchicomula de Serma is considered as traditional agriculture.

All the interviewees produce only one crop cycle per year. The corn varieties grown in this area belong to the Chalqueño race. This corn is grown in association with broad bean (*Vicia faba*), bean, runner bean (*Phaseolus coccineus*), and pea (*Pisum sativum*). Eighty percent of the interviewees use a tractor; 96% use one or several chemical fertilization methods; and 56% use chemical products to control pests and diseases. Sixty percent of the interviewees control weeds mainly by hand and all producers harvest using handpicking methods. Table 4 shows that the total cost of corn production in Chalchicomula de Sesma for the 2020-2021 cycle amounted to \$12,380.00 ha^{-1}.

The average harvest (Q) reached 2,456 kg ha^{-1} and 92% of the harvest is mainly sold to intermediaries. The selling price (P) is \$5.00 kg^{-1}. The TI amounted to \$12,280.00 ha^{-1}; however, only a fraction of the harvest is sold. In average, this fraction amounted to 2,128 kg ha^{-1}, which means that the SR of the 2020-2021 cycle was just \$10,640.00 ha^{-1}. Consequently, the OC for that period only amounted to \$1,640.00 ha^{-1} (Table 5).

Table 4. Total cost of corn production in Chalchicomula de Sesma, for the 2020-2021 cycle.

Activity (\$ ha^{-1})	Fixed Costs	Variable Costs	Cost per Activity
Land preparation	1,200.00	0.00	1,200.00
Sowing	2,450.00	0.00	2,450.00
Fertilization	900.00	1,260.00	2,160.00
Pest and disease control	900.00	970.00	1,870.00
Weed control	450.00	0.00	450.00
Support labor	1,800.00	0.00	1,800.00
Harvest	2,450.00	0.00	2,450.00
TOTAL	10,150.00	2,230.00	12,380.00

Table 5. Corn production income in Chalchicomula de Sesma for the 2020-2021 cycle.

Harvested Corn (Q)	Selling Price (P)	Total Income (TI)	Average Amount of Sold Corn	Sales Revenue (SR)	Self-consumption Opportunity Cost (OC)
2,456 kg ha^{-1}	5.00 \$ kg^{-1}	12,280.00 \$ ha^{-1}	2,128 kg ha^{-1}	10,640.00 \$ ha^{-1}	1,640.00 \$ ha^{-1}

Tequila, Veracruz

According to this research, the total average size of the production units in the municipality of Tequila, Veracruz is 0.56 ha and 64% of the interviewees own the plot of the *milpa*. However, in many cases, the plot used for the *milpa* is rented. Only 6 crops were mentioned by the interviewees and corn represented 55.2% of the arable land. For the purposes of this research, corn production in Tequila is considered as subsistence agriculture.

The corn grown in this area belongs to the Coscomatepec race and it grows in association with bean, runner bean, squash, *cempasúchil* (*Tagetes erecta*), and smooth pigweed (*Amaranthus hybridus*) (grown). Chemical fertilization methods are used by 58.5% of the interviewees; most of them do not control pest or weeds. All of them harvest using handpicking methods. Table 6 shows that the total cost of corn production in Tequila for the 2020-2021 cycle amounted to \$7,350.00 ha^{-1}.

The average harvest (Q) reached 964 kg ha^{-1}; 100% of the harvest is used for self-consumption. The estimated selling price (P) in this study is \$7.00 kg^{-1}. The estimated TI is \$6,748.00 ha^{-1}; however, the harvest is not sold, which means that the sales revenue (SR) for the 2020-2021 cycle was \$0.00, leaving the OC at only \$6,748.00 ha^{-1} (Table 7).

Table 6. Total cost of the corn production in Tequila for the 2020-2021 cycle.

Activity (\$ ha^{-1})	Fixed Costs	Variable Costs	Cost per Activity
Land preparation	2,390.00	0.00	2,390.00
Sowing	880.00	0.00	880.00
Fertilization	520.00	980.00	1,500.00
Pest and disease control	130.00	0.00	130.00
Weed control	650.00	0.00	650.00
Support labor	780.00	0.00	780.00
Harvest	1,020.00	0.00	1,020.00
TOTAL	6,370.00	2,230.00	7,350.00

Table 7. Corn production income in Tequila for the 2020-2021 cycle.

Harvested Corn (Q)	Selling Price (P)	Total Income (TI)	Average Amount of Sold Corn	Sales Revenue (SR)	Self-Consumption Opportunity Cost (OC)
964 kg ha^{-1}	7.00 \$ kg^{-1}	6,748.00 \$ ha^{-1}	0 kg ha^{-1}	0.00 \$ ha^{-1}	6,748.00 \$ ha^{-1}

Profitability Analysis

Considering the productive data of corn grown under *milpa* production systems in each study area, an important difference in the CBR obtained was recoded. This difference matches the productive, technological, investment, and priority capacities of each farmer group that develops every version of the agroecosystem in question (Table 8).

For example, the modern agriculture production system used in Villa de Zaachila, Oaxaca had the highest material and labor investment (\$13,650.00 ha^{-1}); nevertheless, the said expenditure was compensated by the economic benefits (\$23,429.27 ha^{-1}). This amount doubles the initial investment.

Meanwhile, in the scenarios with highest limitations regarding investment, installed capacities, and availability of materials, the productive results were not favorable. Regarding traditional agriculture in Chalchicomula, the total cost of investment (\$12,380.00 ha^{-1}) was similar to the investment of the modern agriculture system, as a result of the use of machinery, labor, and even a minimum amount of agricultural material. Consequently, a considerable production volume was obtained (2,456 kg ha^{-1}). However, the uneven commercial relations with the intermediaries favored low selling prices, reducing their incomes (\$12,280.00 ha^{-1}).

Finally, the case of the subsistence agriculture in Tequila shows a completely different view, as a consequence of the importance given to coffee as main source of income, replacing the *milpa* production system which becomes a complementary and specific self-consumption activity. Therefore, the *milpa* is only an afterthought, taking up "spare" materials and labor from the coffee crops. This situation accounts for the small areas used (0.56 ha) and the low investment spent (\$7,350.00 ha^{-1}) in the *milpa*.

In synthesis, analyzing the productive nuances of the production systems is fundamental. However, this analysis must simultaneously take into account the agricultural, ecological, social, cultural, political, and economy aspects of the regional adaptations. Subsequently, these adaptations must be compared in order to determine their optimal modifications, aimed to the economic benefit of farmer families. These adaptations must also enable the growth of corn crops within an economic model, despite harsh conditions —such as production costs, uneven commercial relations, and low installed capacity, among others.

Table 8. Investment and profit of 1 ha of corn in Villa de Zaachila, Chalchicomula de Sesma, and Tequila for the 2020-2021 cycle.

Concept (\$ ha^{-1})	Villa de Zaachila	Chalchicomula de Sesma	Tequila
Total Production Cost	13,650.00	12,380.0	7,350.00
Yield	2,257	2,456	964
Selling Price	10.00	5.00	7.00
Total Income	23,429.27	12,280.00	6,748.00
Gross Profit	9,779.27	−100.00	−602.00
CBR	1.72	0.99	0.92

The scenarios described above show the diversity of the ecosystems, weather, human resources, capital availability, social relationships, and exploitation logics, regarding corn grown in Mexico, whether in association or as monoculture. Unsurprisingly, models with highest investment, labor availability, and technologies also achieve the highest harvest profits, while the traditional systems which operate in uneven contexts do not only fail to obtain profits, but also compromise food safety.

The *milpa* production system is the guiding principle of the farming agriculture production systems. It is based on the pluriactivity and the multiple use of natural resources, in order to benefit the families and the communities. To unite the said factors, the *milpa* constantly undergoes adaptations, modifications, and substitution of its constituting elements, with the aim to provide the highest possible benefits, even under low or null profit scenarios of its main crop (corn).

CONCLUSIONS

The contrasting modifications in the structure, function, and logic of the *milpa* for the 2020-2021 cycle resulted in a profitable agroecosystem in Villa de Zaachila; however, this system was not profitable in Chalchicomula de Sesma and Tequila. The socioecological factors surrounding the *milpa* are determinant to identify modifications in structure (size, density, crop association), function (the relationship of corn with other associated crops, weeds, and insects) and logic (trade or self-consumption). Nevertheless, these modifications in the *milpa* did not necessarily result in the highest profitability model for an exclusive corn production model for each context.

The result of this study back up the claim that farmers' logic is the basis of corn cultivation, including work, mutual help, experience, deep-rooted connection with the land, food, traditions, family organization, daily life, conservation practices, agricultural lore, and customs. Therefore, rather than provide a monetized economic benefit, corn production meets daily consumption needs. The *milpa* must be understood beyond the plot concept. The *milpa* is a paradigm of the farmers' life and organization, which involves technological learning and innovation, and it goes against the capital tide: *milpa* is community.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Cost analysis of *Agave potatorum* Zucc, produced *in vitro* by direct organogenesis

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ABSTRACT

Objective: To demonstrate the efficiency and profitability of temporary immersion bioreactors compared to propagation in gelled media.

Design/Methodology/Approach: *Agave potatorum* seedlings were introduced to the *in vitro* system in order to compare their productivity, multiplication rate, and propagation time. An investment project was carried out considering the equipment depreciation, but without considering the construction of the property. Based on these premises, the cost of production per plant was evaluated with a goal of 500,000 seedlings in mind.

Results: A 3% contamination was recorded when the *in vitro* system was first introduced. The semi-solid multiplication rate was 4 shoots per explant and 18 shoots per explant in bioreactors, both at 30 days of incubation. A 10% handling loss was taken into consideration. The production cost was US$0.16 (MNX$3.20) for gelled media and US$0.09 (MNX$1.80) for propagation in temporary immersion bioreactors. The Internal Rates of Return for gelled media and for propagation with bioreactors were 2.33 and 3.75, respectively.

Study Limitations/Implications: The study does not take into consideration the construction of the property, although it does consider equipment depreciation.

Findings/Conclusions: Thanks to scientifical and technological development, the use of biotechnological tools is becoming more profitable every day. These technologies are already available to be transferred and this kind of research demonstrates its profitability, highlighting the potential establishment of technology-based companies.

Keywords: Biofactory, agave used for mezcal, profitability, direct organogenesis.

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INTRODUCTION

All over the world, research-focused plant tissue culture laboratories have developed a series of protocols for the propagation of various plant species. Private laboratories (biofactories) use these protocols as a basis for massive plant propagation but consider parameters that research centers do not take into consideration —*e.g.*, the multiplication rate, quality, propagation time, and production costs of these plants, as well as the workforce involved. Likewise, few studies have analyzed production costs and the profitability of these techniques in different plant species (Alamilla-Magaña *et al.*, 2019; Amine Marzri *et al.*, 2021; Criollo-Chan *et al.*, n.d.).

Tobalá spp. (*Agave potatorum* Zucc.) is used to produce mezcal and is currently threatened by unregulated use. These wild plants reproduce through seeds and its populations are being wiped out, along with their genetic variability, since, being non-orthodox plants, their seeds have a maximum viability of 24 months (Enríquez del Valle *et al.*, n.d.; Langlé Argüello *et al.*, November 30 and December 1; Pérez de León *et al.*, 2020).

The *in vitro* multiplication of *Agave potatorum* can be a tool to achieve the massive multiplication that the mezcal market is demanding and this methodology can also be used to repopulate wild plantations. Currently various authors report propagation protocols in semi-solid media with average rates ranging from 6.9 to 14.5 shoots per explant (Aguilar-Jiménez *et al.*, 2018; Domínguez Rosales *et al.*, 2008; Pérez De León, 2022). However, there are very few reports on the propagation of *A. potatorum* using temporary immersion bioreactors (TIB). The present study provides an estimation of the production costs of *Agave potatorum* by direct organogenesis using gelled media and liquid media with twin-flask type TIBs, designed by Escalona *et al.* (1999).

MATERIALS AND METHODS

Process description

The multiplication process by direct organogenesis is based on the use of Tobalá agave meristems and includes the following stages: induction of adventitious shoots, shoot multiplication, shoot elongation, and acclimatization. All experimental data were taken from Pérez De León (2022). Figure 1 shows the flow diagram of the direct organogenesis process.

Induction of adventitious shoots

The study began with fifty *Agave potatorum* meristems, which were subsequently multiplied to obtain the initial amount (500,000 seedlings) and to estimate the production costs (Table 1). Explant disinfection was performed as reported by Aguilar-Jiménez *et al.* (2018). The explants were washed with water and Tween 20 (Cat. 822184, Merck). Subsequently, the explants were put inside a laminar flow hood for 3 minutes in a 70% (v/v) ethanol solution. At the end of that period, they were immersed in a 10% aqueous solution of commercial sodium hypochlorite (Cloralex®) for 15 minutes. Finally, they were rinsed three times with sterile distilled water and the tender leaves covering the meristem were removed until an ≈1-cm explant was obtained. The resulting explants were seeded in a MS medium (Murashigue and Skoog, 1962, Cat. MSP01, Caisson Labs) —supplemented with 3% sucrose (Cat. S011, Caisson Labs) and 0.3% TC Gel (Cat.PTP02, Caisson Labs)— and incubated at 25±2 °C for 30 days with a 16h/8h light/dark photoperiod, in order to evaluate the sterility of the explants. Subsequently, contaminant-free explants were planted in a MS medium (Cat. MSP01, Caisson Labs) supplemented with 2 mg L^{-1} of benzylaminopurine (BA, Cat. B800, Phytotechnology) and 4% sucrose (Cat. S011, Caisson Labs).

Figure 1. Flow diagram of the propagation process by direct organogenesis.

Table 1. Induction and multiplication process in production systems with gelled media and liquid media (temporary immersion bioreactors: TIB).

Induction		Multiplication		
SS		**PHASES**	**SS**	**TIB**
Initial meristems	100	Initial explants	17500	8000
Induction cycles, 60 days	5	Multiplication cycles	11	5
Explants obtained at the end	149,778	Final explants obtained	500,500	608,000

Adventitious shoots multiplication

Organogenic cultures were grown in a MS medium (Cat. MSP01, Caisson Labs) supplemented with 3% sucrose (Cat. S011, Caisson Labs), 10 mg L^{-1} BA (Cat. B800, Phytotechnology), and 0.3 mg L^{-1} indole acetic acid (IAA, Cat. I885, Phytotechnology). The pH was adjusted to 5.7 and it was sterilized in an autoclave at 120 °C, at 1 atm pressure for 20 min. They were incubated at 25±2 °C for 30 days with a 16h/8h light/darkness photoperiod, at 32 μmol s^{-1} m^{-2}. Shoots longer than 4.0 cm, with at least three leaves, were chose for acclimatization.

Seedling acclimatization

The acclimatized seedlings had at least three leaves and were at least 10 cm long. They were cultivated in polystyrene trays with 200 cavities, with a substrate made of a 50:50 mix of peat moss (Sushine mix #3) and greenhouse soil. They were placed in a shade-house with 50% shade cloth for 60 days at room temperature. The first seven days, the seedlings were irrigated every day with 50-L/min sprinklers for 30 min; subsequently, they were irrigated every two days under the same conditions.

Cost Calculation

At the beginning of the multiplication, the induction process, and the work to obtain the required number of shoots took 5 months. A goal of 500,000 seedlings per year was established; therefore, the TIB was carried out in 5 cycles of 30 days and, in the case of semi-solid media, it was carried out in a 11-month period. The components considered in the cost analysis are included in the Calculation Report. A Caisson Labs® culture medium was purchased. The remaining products (sodium hypochlorite, ethanol, Kraft paper, aluminum foil, sanitary and cleaning products, among others) were purchased in local markets. The calculation of the personnel salary was based on the number of hours worked and the Mexican salary tabulator as of January 2021. The electricity, water, and fuel costs were calculated according to their use in the production cycles. Building and infrastructure were not considered, since the study was carried out in functioning laboratories; however, equipment depreciation was considered and an investment project was carried out for this purpose. Microsoft Excel 2016 was used for all calculations.

RESULTS AND DISCUSSION

Induction of adventitious shoots

After two months of culture, 3% of the explants were eliminated due to contamination, mainly by fungi. Fifty percent of the culture media contaminated by bacteria were saved with 50 ppm of silver nanoparticles. During the first three months, adventitious shoots appeared in the meristems. For the medium changes, the same number of flasks were used and, after four months, the first division of the adventitious shoots took place. Afterwards, they multiplied for four more cycles, until the amount necessary for multiplication in the different systems was reached. Thirty 940-ml TIB were used to place 600 initial shoots and 175 initial explants were used to carry out the propagation projection in TIB. The goal was reached in five cycles of 30 days. In a semi-solid medium, the goal was reached in eleven cycles of 30 days. A 10% contamination was attributed to the handling by the operators (Table 1). For the induction and multiplication stage —before scaling up and obtaining 108,200 adventitious shoots—, an average of 416.1 L of culture medium was used.

Multiplication of adventitious shoots

The number of adventitious shoots produced by organogenic culture was 4 for semi-solid media and 18 for liquid media in TIB. The multiplication goal was 500,000 seedlings; this amount was used for the projections and cost analysis of both systems. For the semi-solid medium, 500,500 seedlings were projected using a 1,925-L medium volume during 11 months. For the TIB, 608,000 seedlings were projected, using a 1,200-L medium volume for 5 months. For both systems, a 10% loss due to contamination and a 10% loss due to acclimatization were considered, in the case of the investment project and the determination of production costs. Various studies about agaves report multiplication rates. Monja-Mio Kelly *et al*., (2021) report a maximum multiplication rate of 6.23 shoots/explant, using temporary immersion bioreactors in *Agave angustifolia*. Aguilar-Jiménez *et al*., (2018) reports a multiplication rate of 14.5 shoots of *Agave potatorum* per explant in semi-solid medium. The results of this work surpass those findings, mainly as a consequence of the protocol and the TIB type used.

Seedling acclimatization

After the multiplication phase in both systems was over, the seedlings were sent directly to acclimatization. Arysta® Raizal 400 was used to induce the root in the acclimatized seedling. A 50:50 mixture of peat moss (Sphagnum Peat Moss-Premier Tech Horticulture) and soil from greenhouses was put into polystyrene trays with 200 cavities. There was a 95% survival rate of seedlings from semi-solid medium and 98% of seedlings from TIB. Domínguez Rosales *et al.* (2008) report that, after 30-45 days of acclimatization in *A. potatorum* seedlings, 73% of the seedlings survived. Monja-Mio *et al.* (2020) reported 73% survival of *A. tequilana* seedlings.

Cost calculation and investment project

Initially, the costs of the culture medium per liter used in both systems were compared (Table 2). The gelling agent represents 48.3% of the total cost of the culture medium; therefore, using only liquid media in the TIB reduced production costs. An investment project was developed to carry out the cost analysis, with a fixed asset of US$57,039.71, a deferred asset of US$500.75, and a working capital of US$48,218.16. Table 3 shows the Calculation Report used to carry out the investment project and establish the financial indicators and the cost of production per plant.

The following results were obtained from the cost analysis and the investment project: the cost of producing *in vitro* seedlings using the semi-solid medium was US$0.16 and using TIB was US$0.09 (both including equipment depreciation). In both systems, the

Table 2. Cost of culture medium and percentage in relation to cost.

Description	Cost per semi-solid liter (US$)	Cost per liquid liter (US$) 1	Percentage of total cost (%)
Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 1 kg Brand: CAISSON	0.42	0.42	14.3
Gellex® Gellan Gum. Presentation: 500 g., Brand: CAISSON	1.41	-	48.3
6-Benzyl aminopurine (BAP) 25g., Brand: CASSION	0.03	0.03	1.1
Sugar (Chedraui) 2 kg.	0.05	0.05	1.6
Distilled water	1.02	1.02	34.7
Total cost	2.93	1.52	100

The prices of the products were obtained on 02/09/2021. The exchange rate ($19.97 Mexican pesos per US dollar) for that day was checked with Banco de México.

Table 3. Financial indicators of the investment project for the *in vitro* propagation systems of *Agave potatorum* Zuuc.

INDICATORS	SS	TIB
Net Present Value (NPV)*	US$ 375,551.03	US$ 485,127.20
Internal Rate of Return (IRR)	185.39%	237.03%
Benefit/Cost Ratio (BCR)	2.33	3.75

*The indicators were calculated in Mexican pesos and converted to US dollars at a rate of $19.97 Mexican pesos per US dollar.

seedlings are *in vitro* without acclimatization. A selling price of US$0.5 was established for both systems, which indicates that they have a good profitability. In order to corroborate these findings, the financial indicators of the investment project were calculated for both systems (Table 4). The Benefit/Cost Ratio (BCR) for semi-solid media (SS) and for TIB were 2.33 and 3.75, respectively. For every dollar invested, 1.33 is recovered for the SS

Table 4. Calculation report for the temporary immersion bioreactors (TIB) and Gelled Media (SS) system. Price in US dollars, with exchange rate of $19.97 Mexican pesos per US dollar, quoted on 02/09/2021.

TIB				SS			
CONCEPT	QUANTITY	UNIT PRICE	TOTAL	CONCEPT	QUANTITY	UNIT PRICE	TOTAL
Scalpel #3	5	$23.72	$118.60	Scalpel #3	10	$23.72	$237.21
Scalpel #4	5	$23.72	$118.60	Scalpel #4	10	$23.72	$237.21
Scalpel blades 24 c/100 pcs	10	$11.07	$110.67	Scalpel blades 24 c/100 pcs	20	$38.86	$777.21
Scalpel blades 11 c/100 pcs	10	$11.07	$110.67	Scalpel blades 11 c/100 pcs	20	$38.86	$777.21
Surgical mask c/50 pcs	14	$6.91	$96.75	Surgical mask c/50 pcs	28	$6.91	$193.49
Surgical cap c/100 pcs	7	$14.97	$104.81	Surgical cap c/100 pcs	14	$14.97	$209.61
Gellex® Gellan Gum. Presentation: 500 g Brand: CAISSON (G017-500GM)	1	$82.55	$82.55	Gellex® Gellan Gum. Presentation: 500 g Brand: CAISSON (G017-500GM)	14	$82.55	$1,155.66
Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 100 L Brand: CAISSON (MSP09-100LT)	30	$56.73	$1,702.02	Murashige & Skoog with macronutrients, micronutrients, and vitamins Presentation: 100 L Brand: CAISSON (MSP09-100LT)	22	$56.73	$1,248.15
Benzylaminopurine Brand: Phytotechnology (b800-25g)	2	$78.42	$156.84	Benzylaminopurine Brand: Phytotechnology (b800-25g)	1	$78.42	$78.42
pH Buffer	3	$8.01	$24.04	pH Buffer	3	$8.01	$24.04
Potassium hydroxide 250 g	2	$8.01	$16.02	Potassium hydroxide 250 g	2	$8.01	$16.02
Hydrochloric acid 1 l	1	$6.26	$6.26	Hydrochloric acid 1 l	1	$6.26	$6.26
Consumables	1	$751.13	$751.13	Consumables	1	$751.13	$751.13
Work days	336	$10.02	$3,365.05	Work days	336	$10.02	$3,365.05
TOTAL			$6,763.99	TOTAL			$9,076.65

system and 2.75 for the TIB system. This proves the profitability of micropropagation. Likewise, the use of TIB —in addition to improving plant quality— increases at least two times the rate of multiplication and decreases time, vastly increasing the profitability of this type of multiplication. The use of biotechnological methods to decrease the cost of massive multiplication does not only promote the creation of technology-based companies, but also makes these technologies accessible to producers, meeting the demand for this species of agave used in the production of mezcal and helping to prevent the decline of wild populations of the Tobalá agave.

CONCLUSIONS

The use of biotechnological tools becomes more common day by day. Strategies have been developed for the transfer and adoption of plant micropropagation technologies. However, plant tissue culture laboratories are still preferred over biofactories. The difference between laboratories and biofactories is that the latter already use validated protocols and focus on plant quality, as well as the efficiency and (above all) the profitability of the processes. Consequently, works such as ours are required to start the transfer of technologies and encourage their adoption by entrepreneurs or by already established companies. This study was able to demonstrate that both processes have good profitability; however, the use of TIB exceeds profitability by almost 1.5 times, demonstrating that the use of this method in this agave species can have a positive impact on the adoption of the technology by the companies. This method is the first proposed for this species; therefore, it is innovative and can be used for transfer to different entities. A large amount of raw material can be exploited in other market niches —*e.g.*, ornamentals, biomass production, sugars, etc.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Population fluctuation of the pineapple mealybug in two pineapple (*Ananas comosus* [L.] Merr.) varieties in Huimanguillo Tabasco, Mexico

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ABSTRACT

Objective: To identify the pineapple mealybug *Dysmicoccus* spp. and determine its prevalence and seasonal distribution in Huimanguillo Tabasco, Mexico.

Design/Methodology/Approach: This study was carried out in two pineapple producing localities in the municipality of Huimanguillo, Tabasco. The varieties studied were MD2 and Cabezona. The seasonal distribution and infestation percentage was determined in both localities and for both varieties from January to June 2021. Forty plants were randomly sampled per month in a 4-ha area per locality. Roots, stems, basal leaves, and fruits were examined at the plant level.

Results: The highest abundance of pineapple mealybugs (*D. brevipes* sp.) was found in the Cabezona variety. *D. brevipes* is mainly located in 85.46 and 61.76% of the old leaves of the stem, in the Cabezona and MD2 varieties, respectively. From January to April —when the highest temperatures were recorded—, the population of *D. brevipes* was moderate, while the highest populations were recorded towards mid-June, when the rains began.

Study Limitations/Implications: Climatic variables can affect the evaluation parameters. The immature stages dynamics can limit the interpretation of results.

Conclusions: *D. brevipes* is mostly distributed in the lower parts of the plant. To determine the population fluctuation, predict the total number of insects per plant, and establish their temporal and spatial distribution, the entire production cycle must be considered, as well as the climatic parameters, in order to develop a targeted control strategy on dates of moderate abundance.

Keywords: Pineapple mealybug, seasonal distribution, Huimanguillo.

INTRODUCTION

The cultivation of pineapple (*Ananas comosus* (L.) Merr.) is of great importance worldwide due to the high income it generates. In Mexico, the largest cultivation areas are found in Veracruz, Oaxaca, and Tabasco. In Tabasco, the municipality of

Image by Igor Ovsyannykov at Pixabay

Huimanguillo has the largest area for cultivation. In many localities small producers work in areas of up to 4 ha. For their part, commercial plantations with more than 300 ha use intensive technology in a protected environment, with shade cloth and plastic mulch. The volume of Mexican pineapples exported to the US increased from 25,000 to 100,000 tons/year (Torres *et al.*, 2018). Meanwhile, various environmental and phytosanitary factors have caused a decrease in yields. The management of the negative effect of epidemic attacks caused by pests requires an appropriate solution. The pineapple mealybug (*Dysmicoccus brevipes* (Cockerell)) is one of the insects that has a high impact on the pineapple crop: not only does it cause direct damage by sucking the sap from the plant, but it also is the vector of the virus disease called mealybug wilt of pineapple, which can generate losses of up to 40% of production (Martínez *et al.*, 2006; Bertin *et al.*, 2013). This pest has a large number of hosts. In the Huimanguillo Tabasco region, the taxonomy of *Dysmicoccus* spp., its population fluctuation, and its distribution in plants have not been determined. Therefore, the present study was developed with the objective of identifying the species and to determine the population fluctuation and spatial distribution of *Dysmicoccus* spp. in two pineapple varieties grown in the municipality of Huimanguillo, Tabasco, in order to determine the bases for the agroecological management of this pest in pineapple cultivation.

MATERIALS AND METHODS

Location of the study area

The field work was carried out in two pineapple production areas in the municipality of Huimanguillo, Tabasco, Mexico, where the MD2 variety and a variety locally known as “Cabezona” are grown. Sampling was carried out for six continuous months (January-June 2021), from the beginning of sowing, although fruit samples were also collected during the harvest stage.

El Milagro. The ranch known as “El Milagro” produces pineapples with both conventional (300 hectares) and organic (20 hectares) methods. The latter area was established in 2019. This ranch is located on the way to Estación Zanapa, km 8 S/N, in Ejido Encomendero, municipality of Huimanguillo, Tabasco. It is located at a latitude of 17° 43’ 15.63” and a longitude of −93° 41’ 37.49”, at an altitude of 20 m.a.s.l. Temperatures range from 20 °C to 37 °C, rainfall varies between 34 and 112 mm, and relative humidity fluctuates between 67 and 88% (Weatherspark, 2021). The soils of this region are Cutaneous Umbric Acrisol (Salgado *et al.*, 2017).

La Esperanza. Pineapple production of the variety locally known as “cabezona” was identified in this area. Cabezona is a triploid with fruits of up to 5 kg, large plants, and many thorns on the leaves (Bartholomew & Rohrbach, 2002). This town is located in Ejido La Esperanza, municipality of Huimanguillo, Tabasco, at a latitude of 43° 11’ 43.8” and a longitude of 19° 48’ 2.7”, at an altitude of 24 m.a.s.l. Temperatures range from 20 °C to 37 °C, precipitation varies between 34 and 112 mm, and relative humidity fluctuates between 67% and 88% (Weatherspark, 2021). The soil belongs to the Cutaneous Umbric Acrisol type (Salgado *et al.*, 2017).

Sample processing and identification of the pineapple mealybug

Whole samples of pineapple plants with mealybug individuals were taken to the Laboratorio de Control Biológico of the Colegio de Postgraduados, Campus Tabasco, Cárdenas, Tabasco, where they were identified. The pineapple mealybug specimens were sent for their identification to Mr. Héctor González Hernández (ScD), from the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of Mexico.

The pineapple mealybugs were processed following the mounting technique for scale insects (Hemiptera: Coccomorpha) developed by Kosztarab (1963). This method is an adaptation, with minor changes, of the procedure used for micro-insect mounting established by the U.S. Department of Agriculture (USDA).

H. González Hernández identified the pink pineapple mealybug, using the keys developed by Williams and Granara de Willink (1992).

Seasonal distribution of *D. brevipes* in two varieties of pineapple from two locations in Huimanguillo Tabasco, Mexico

To determine the seasonal distribution of *D. brevipes* in the two locations and two pineapple varieties, a random destructive sampling was used, in the form of a diagonal line, according to a method modified by González Hernández *et al.* (1999). This method consisted of taking 10 plants/ha, sampling one plant every 25 m, with 10 m between double rows. In total, 40 plants were sampled in the 4 ha of the study's sampling area. The mealybugs present on each uprooted plant were counted with the aid of a magnifying glass. The counts were made directly in: A) roots and underground stems, B) basal leaves of the stem at ground level, and C) fruits (Figure 1).

Analysis of data

To determine the incidence, distribution, and population fluctuation of the pineapple mealybugs (*D. brevipes*) found in the organs of the plants evaluated in both study areas,

Figure 1. Parts of the plants sampled A) roots and underground stems, B) stem leaves at ground level, and C) fruits.

monthly registration was carried out in Microsoft Excel. An analysis of variance was made for each of the evaluated variables, in which the locality/variety and the sampling month were considered as variation sources. Likewise, a multiple comparison test of means was carried out using Tukey's test (SAS, 2012).

RESULTS AND DISCUSSION

The mealybug species detected in the two localities with pineapple plantations in this study was *Dysmicoccus brevipes* (Cockerell) (Hemiptera: Pseudococcidae): the pink pineapple mealybug (Figure 2), a species of cosmopolitan distribution, already reported in pineapple, grasses, and agaves in Mexico (Williams & Granara de Willink, 1992).

In the case of the number of *D. brevipes* adult females in the pineapple stem basal leaves, highly significant statistical differences were found between both localities and varieties ($p<0.0001$). The highest number of pineapple mealybugs was found in Ejido La Esperanza, in the Cabezona pineapple variety, with an overall average of 84 individuals per plant during the 6 months sampled. Meanwhile, in El Milagro ranch, the MD2 variety had a lower number of individuals, with an average of 25 adults of *D. brevipes*. The highest number of pineapple mealybugs detected was 120 adult females per plant in the month of June 2021 for the La Esperanza/Cabezona combination, while the highest number of adult females was recorded with the El Milagro/MD2 combination during the

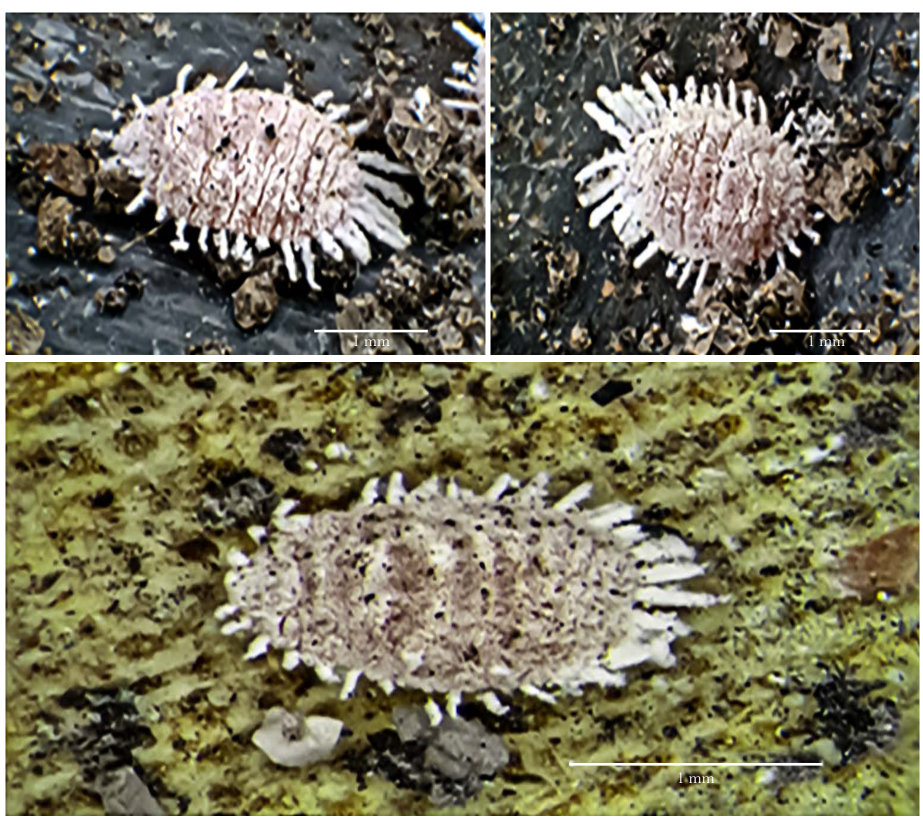

Figure 2. Adult females of *D. brevipes* (photo by H. González-Hernández, 2021).

month of May 2021 (11 pineapple mealybugs per plant); a slight increase was recorded in June (Figure 3 A).

Regarding the presence of *D. brevipes* in the root zone of the pineapple plant, highly significant statistical differences were also found between both locations/varieties ($p<0.0001$), although the population of pineapple mealybugs at the base of the stem was very small compared to the above results. The highest number of *D. brevipes* was recorded by the La Esperanza/Cabezona combination (up to 4 individuals per plant), while the El Milagro/MD2 combination registered the highest number of individuals in May 2021, with an average of 2 pineapple mealybugs (Figure 3 B).

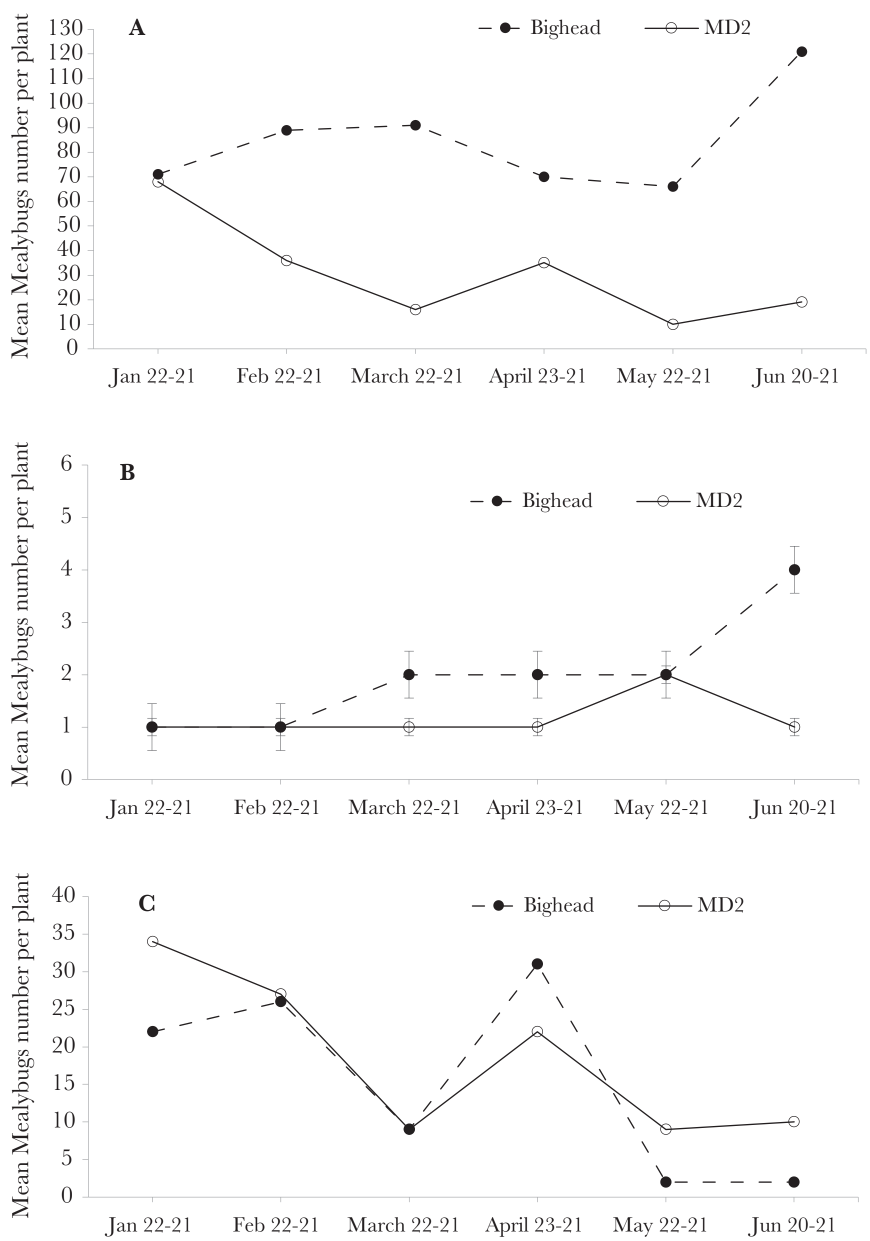

Figure 3. Prevalence of *D. brevipes* in A: Stem and basal leaves, B) roots and C) fruits of pineapple, from January 22, to June 20, 2021, in two areas and varieties in Huimanguillo Tabasco, Mexico.

However, both locations/varieties also show significant statistical differences ($p<0.05$) regarding the number of *D. brevipes* individuals in the fruit. Contrary to what happens in the root and stem leaves, in MD2 fruits sampled at the El Milagro ranch, the highest number of *D. brevipes* was found in January, with up to 34 individuals of the pineapple mealybugs (Figure 1 C)

Regarding the distribution of *D. brevipes* among the sampled parts of the pineapple plant, a higher percentage of infestation was observed in stem leaves (85.98%), fruit (13.71%), and root (0.013%) of the Cabezona variety in La Esperanza. Meanwhile in El Milagro, the percentage infestation of MD2 was higher in stem leaves (61.76%), fruits (37.55%), and root (0.68%) (Figure 4).

Regarding the seasonal distribution and the infestation percentage by *D. brevipes*, the results indicate that there were significant differences ($p<0.0001$) between the locations/varieties of pineapple: the infestation percentage of the Cabezona variety was higher in Ejido La Esperanza.

The Cabezona variety cultivated in approximately 200 ha of Ejido La Esperanza has a minimal agronomic management, especially regarding *D. brevipes*. In some cases, this management is completely deficient, as a consequence of several criteria, including scarce knowledge of the producer about these problems. For example, pineapple mealybugs and their associated ants are exclusively managed applying insecticides (such as diazinon and chlorpyrifos) once or twice per crop cycle. Insecticides are applied at the beginning of sowing (November-December) and in the middle of the cycle (July-August). Consequently, an increasing trend of *D. brevipes* is observed from February to June in the Cabezona variety. The areas harvested and awaiting destruction for subsequent pineapple plantations lack an adequate management; therefore, they are sources of *D. brevipes* reproduction (Mau and Martín, 1992). Likewise, the edges of the plantations are totally infested with weeds, which could be reservoirs of this pest (Martínez *et al.*, 2006, García De la Cruz *et al.*, 2021).

Meanwhile, the lower abundance of *D. brevipes* in the MD2 variety can be explained in part by aspects of agronomic management. This study was also carried out in the Agrícola San Pablo company. The plantations of this company (including El Milagro ranch) grow

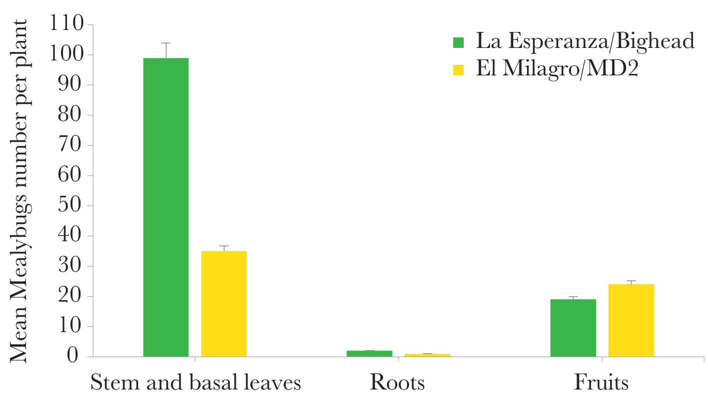

Figure 4. Prevalence and overall distribution of *D. brevipes* in pineapple plants from January to June 2021, in two locations/varieties of Huimanguillo Tabasco, Mexico.

commercial MD2 pineapple and Persian lime. This variety is grown in approximately 287.24 ha. Currently, the company owns 48 plantations more where MD2 pineapple production is carried out in more than 300 ha. Those pineapples are in different stages: development, production, and nurseries. In this ranch, the agronomic management of the plantations is done in a planned and intensive manner, making use of high-tech, since the production is frequently exported. To manage *D. brevipes*, the company carries out sanitation pruning after harvest, applies herbicides such as Paraquat to finish the crop, and destroys and incorporates harvest residues. It also carries out a chemical control program based on class 1B organophosphate insecticides Diazol 50 EW® (diazinon) and class 3B pyrethroids (cypermethrin). These products are applied bimonthly, together with other chemicals; they are regularly applied in the foliar area using a boom sprayer. However, this type of pest management could lead to a scenario where pineapple mealybug becomes resistant to constantly applied insecticides (Venkatesan *et al.*, 2016). Pineapple production will continue to increase in the future and, therefore, looking for eco-friendly management strategies (such as the use of more selective products with low impact on beneficial fauna) must be a priority, within a comprehensive pest management arrangement. These strategies would allow producers to sustainably manage this pest.

The monthly temperature data recorded for the municipality of Huimanguillo from January to June 2021 was 26, 30, 29, 33, 37, 34 °C, respectively (Figure 5). It is inferred that these conditions favor the proper development of *D. brevipes*, since its population does not undergo dramatic changes. Bertín *et al.* (2019) mention that a total of 678.4 degree-days are required to complete the development from the first nymphal instar to the adult stage and that the most favorable temperature for the optimal development of *D. brevipes* is 30 °C.

Regarding the seasonal distribution and the percentage of *D. brevipes* infestation, the results indicate that there were significant differences ($p<0.0001$) between the pineapple varieties. The infestation percentage was higher in the Cabezona variety. This variety shows a vigorous growth and has very large plants and high Fe content in its leaves (Murillo *et al.*, 2019); consequently, the lower parts of the plant (basal stems, old leaves and roots)

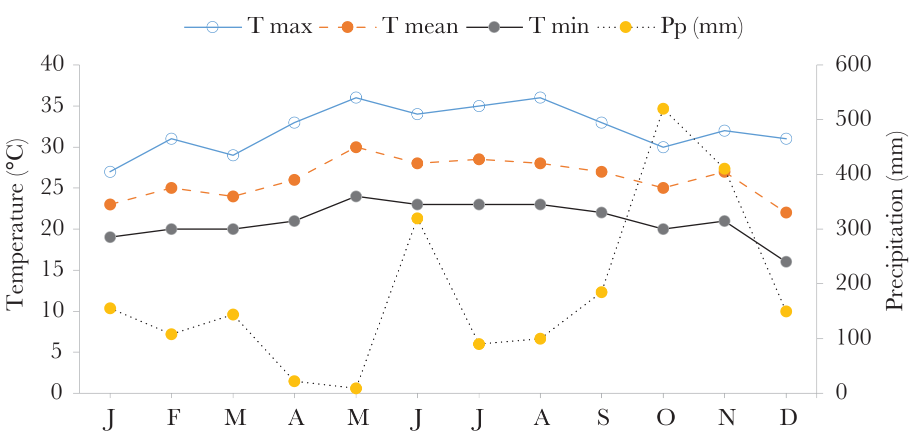

Figure 5. Fluctuation in temperature and rainfall (monthly mean) during 2021, around pineapple fields in Huimanguillo, Tabasco.

receive less light and are more susceptible to pests, which find better conditions for their development (Valdes *et al*., 2019). The lower parts of the plants have a greater number of colonies; the curvature of the old basal leaves protects pineapple mealybugs from light or natural enemies. It also reduces the efficiency of the chemical products applied to control them (Valdes, 2019; Hernández and Peña, 2009). According to Mau and Martín (1992), Ciesla (2000), and Cermeli *et al*. (2002), mealybugs have been detected sucking the sap of any part of the plant; they prefer to attack the base of the leaves of smaller plants. Meanwhile, in older plants, the infestation passes through the stems until it reaches the fruit. This study shows that *D. brevipes* attacks various parts of the plant and the fruit of both varieties. It has medium impact on the MD2 variety, because it can lead to its rejection as an export fruit.

Our findings match those of Valdes *et al*. (2019), who showed that *D. brevipes* feeds on any external part of the plant and that, undoubtedly, prefers to attack the base of the stem and leaves in the semi-underground part, where ant colonies are also found. The seasonal distribution varies notably according to the time of year since, according to Valdés *et al*., (2019), pineapple mealybugs are generally located in the lowest stratum of the pineapple plant during the dry season, while in the rainy season they tend to be found in the axils of intermediate leaves.

CONCLUSIONS

Dysmicoccus brevipes (Cockerell) is found in both localities, both in the MD2 and in the Cabezona varieties. The population of this mealybug species tends to increase towards the beginning of the rainy season, starting in June. This insect feeds on various parts of the plant, with special preference for the lower parts (stem and basal leaves), at the semi-subterranean level. Based on these data, we can infer that the sanitary management aspects of this pest must be directed from the beginning of the plantation, using healthy propagation materials and sustainable agroecological management programs, according to the population levels detected in this study.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Naomi Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch) variety for indoor spaces

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ABSTRACT

Objective: To evaluate the behavior of the Naomi poinsettia variety and its varietal description applying the guidelines of the Union for the Protection of New Varieties of Plants (UPOV).

Design/Methodology/Approach: The variety was generated through the following methods: plant genetic hybridization, individual selection, and use of the grafting technique. The varietal description was based on the document of the Union for the Protection of New Varieties of Plants (UPOV). A completely randomized experimental design with 10 replications was used. Vegetative and reproductive characteristics of the plant were recorded. The data was subjected to an analysis of variance and Tukey's mean comparison test ($P \leq 0.05$).

Results: The Naomi variety is tall with intermediate width and branching. It has a very intense green color in the middle third of the stem. It has oval-shaped leaves of intermediate size, with a rounded base, and without curvature in the main nerve. Its elliptical bract does not have a torsion, but it has an intermediate roughness between its nerves.

Study Limitations/Implications: The variety is propagated asexually via cuttings and the plants from which the cuts are made are preserved as mother plants. It needs adequate nutrition and light and temperature conditions that hinder floral differentiation.

Findings/Conclusions: The new poinsettia material has phenotypic attributes demanded by the market. The varietal description indicates that it is comparable with other INIFAP varieties and with commercially available varieties. This plant can be considered a competitive candidate for the diversification of the offer of poinsettia plants in the Christmas season.

Keywords: Bract, leaf, archetype.

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INTRODUCTION

Euphorbia pulcherrima Willd. ex Klotzsch —known as nochebuena or poinsettia— is considered a symbol of Christmas around the world. Mexico has a long tradition of growing this ornamental species. Every year, several municipalities of the state of Morelos have been identified as major producers of finished plants; the commercialization of the poinsettias is carried out in the producers' own nurseries.

In Mexico, the annual demand amounts to approximately 20 million poinsettia plants (SIAP, 2018), which are sold in 3–12-inch containers. The whole production is sold from November to mid-December, generating 718 million 372 thousand Mexican pesos. This sector uses a large amount of labor in the management of the crop itself, in post-harvest preparation, and in marketing. Worldwide poinsettia production is close to 500 million plants and is experiencing a constant increase in Europe, Asia, and North America, due to its economic, aesthetic, and commercial potential (Vilperte *et al.*, 2021).

In Mexico, the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) has implemented the Programa de Mejora Genética de Nochebuena in the Campo Experimental Zacatepec, Morelos (Canul-Ku *et al.*, 2021). The genetic improvement criteria applied are essentially focused on the characteristics of the leaf, bract, and plant architecture (Canul-Ku *et al.*, 2017). A national germplasm with a broad genetic base (Canul *et al.*, 2013) has provided the bases for the strategies that have been followed: using manual crossing of materials with the best aesthetic attributes demanded by the market (Canul-Ku *et al.*, 2017); reducing plant height; improving the branch growth capacity (Canul-Ku *et al.*, 2018); and using grafting to improve the overall plant architecture (García-Pérez *et al.*, 2017).

The objective of this study was to evaluate the behavior of the Naomi poinsettia variety in the state of Morelos. A local producer cooperated in this research, in which the varietal description was carried out applying UPOV guidelines.

MATERIALS AND METHODS

The Naomi variety was generated in the Campo Experimental Zacatepec (INIFAP), located in Zacatepec de Hidalgo, Morelos, Mexico, at 18° 39' 16" N and 99° 11' 54" W and at an altitude of 910 m. The plant genetic improvement methods used in the generation process were hybridization, individual selection, and the grafting technique.

In 2011, the simple manual crossing between the NIS2P6×NFMOR parents was carried out applying the technique generated by Canul-Ku *et al.* (2015). In 2012, the F_1 progeny was evaluated, and the most promising plants were selected based on their aesthetic attributes; the plants were propagated asexually through cuttings in 2013. Subsequently, four cycles of individual selection were carried out in the 2014-2017 period. In 2018, the new variety was grafted onto a commercial variety using the wedge technique (García-Pérez *et al.*, 2017). The resulting progeny was increased in number by asexual propagation (cuttings) and was evaluated in 2019. This form of propagation maintains the genetic and phenotypic identity of the variety, since the characteristics of the next clonal generation will be stably inherited and maintained in the cuttings used to establish new crop cycles (Islam *et al.*, 2013).

The varietal description was carried out in 2020 applying the guidelines of the Union for the Protection of New Varieties of Plants (UPOV, 2008) in Tetela del Monte (18° 58' N, 99° 15' W, and 2000 m.a.s.l.), municipality of Cuernavaca, state of Morelos, Mexico. For this purpose, 10 plants of the new variety were established in 6-inch pots and the Alhely and Beatriz varieties were used as controls for the varietal comparison. The crop was managed through the technological package generated by INIFAP (García *et al.*, 2017). It should be pointed out that growth regulators were not applied, in accordance with UPOV guidelines. In the bract pigmentation stage (specifically with three open cyathia), 53 characteristics were documented applying the UPOV criteria (2008).

In addition, 11 vegetative and reproductive characteristics of continuous distribution were recorded. This information was used to perform an analysis of variance and Tukey's mean comparison test ($P \leq 0.05$) with the statistical analysis software SAS version 8.1 (SAS, 2000).

RESULTS AND DISCUSSION

The generation of the Naomi variety was a 10-year process. The crossing of the parents and the evaluation of the F_1 progeny was carried out in the Campo Experimental Zacatepec, while the selection and description were carried out in Tetela del Monte —one of the areas with the highest production in the state of Morelos, located in the municipality of Cuernavaca.

Behavior

As shown in Table 1, the morphophenotypic response of Naomi was comparable to that of other varieties generated by INIFAP: bract length, leaf width, and leaf petiole length were statistically similar. Naomi's bract width was equal to the Alhely variety.

The aesthetic and commercial value of poinsettia lies in its architecture, the compact size of the plant, its quantity, and the color of the bract. Naomi had the highest number of branches (Table 1), each one representing a potential bract. Overall, a higher number of bracts entails greater acceptance among the consumers and in the market.

Naomi had an intermediate response in terms of plant height, stem diameter, and bract petiole length. However, it had the lowest leaf length (Table 1).

Table 1. Comparison of the means of the evaluated characteristics of the Naomi poinsettia variety and two controls.

Character	Alhely	Beatriz	Naomi	DMSH	CV%
Plant height (cm)	59.92 a	43.88 c	48.94 b	4.17	7.39
Stem diameter (mm)	14.53 a	10.71 c	12.51 b	1.80	12.93
Leaf length (cm)	12.14 a	12.12 a	9.43 b	1.41	11.38
Leaf width (cm)	7.97 a	7.73 a	7.02 a	1.21	14.47
Leaf petiole length (cm)	4.23 a	3.86 a	3.56 a	0.68	15.91
Number of branches	6.3 b	6.7 b	9.1 a	1.84	22.63
Bract width (cm)	5.66 a	4.3 b	5.83 a	0.74	12.74
Bract length (cm)	9.51 a	10.22 a	9.94 a	1.18	10.78
Bract petiole length (cm)	2.03 b	2.56 a	2.36 ab	0.51	19.91
Bract canopy width (cm)	22.03 b	29.2 a	23.38 b	3.03	11.07
Cyathium diameter (cm)	3.45 ab	5.6 a	3.06 b	2.25	21.6

DMSH=Honest minimal significant difference, CV=Coefficient of variation, Values with different letters within rows indicate significant differences (Tukey, $P \leq 0.05$).

Characteristics

The Naomi variety is a tall plant with intermediate width and branching. It has a very intense green color in the middle third of the stem. The intensity of anthocyanin pigmentation in the middle third and upper third of the stem is likewise strong. It has oval-shaped leaves of intermediate size, with a rounded base, and without curvature in the main nerve. Its elliptical bracts do not have a torsion, but they have an intermediate roughness between their nerves. According to the color chart of the Royal Horticultural Society (RHS, 2007), the color of the upper part of the bract is 53A and that of the lower part 53B. The width of the top is narrow. The cyathium glands are small, yellow, and not deformed. According to its response to the change of season (approximately, September 21), it is considered a late cycle variety (Figure 1).

The production of Naomi is feasible in the various states that produce poinsettias in Mexico. The technological management of the variety by the cooperating producers resulted in a good commercial quality of the plant. Meanwhile, the preventive application of pesticides protected the plants from pests and diseases.

The registration of the Naomi variety before the Servicio Nacional de Inspección y Certificación de Semillas (SNICS–SADER) is pending, with the purpose of obtaining the plant patent and its inclusion in the Catálogo Nacional de Variedades Vegetales (CNVV).

The commercial exploitation of the variety is possible through the signing of a licensing agreement. Mother plants are available at the Campo Experimental Zacatepec, in the state of Morelos. To maintain the varietal identity and genetic purity, it is kept in a vegetative form and is propagated vegetatively through cuttings.

Figure 1. Phenotypic attributes of the Naomi variety.

CONCLUSIONS

The new poinsettia material has phenotypic attributes demanded by the market. The varietal description indicates that it is comparable with other INIFAP varieties and with commercially available varieties. The Naomi variety is tall with intermediate width and branching. It has oval-shaped leaves of intermediate size, with a rounded base, and without curvature in the main nerve. Its elliptical bracts do not have a torsion, but they have an intermediate roughness between their nerves.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of annual rainfall and temperature on the selection of habitat and overwintering home range of grassland birds

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ABSTRACT

Objective: To determine the effect of annual rainfall and winter temperature on the habitat and size of the overwintering home range of two grassland birds in a native grassland in northern Durango, Mexico.

Design/Methodology/Approach: Using telemetry techniques during four consecutive winters (2016-2019), we estimated the size of the home range (HR; by the Kernel method) of *Centronyx bairdii* and *Ammodramus savannarum*. Likewise, the coverage of grassland, scrubland, bare ground, dead vegetation, and animal excrement was estimated and the seeds available in the soil (biomass, g m^{-2}) of the habitat were counted. We correlated these dependent variables with non-parametric statistics, to the minimum and maximum annual rainfall (mm) and temperature (°C) of the site.

Results: Grassland sparrows used sites with equitable cover of grass, herbaceous plants, shrubs, bare ground and others (Kruskal-Wallis, $p \leq 0.05$). Overall, annual rainfall has no effect on structure and vegetation cover. The HR was negatively correlated (Spearman, $p \leq 0.05$) with the annual rainfall for *C. bairdii* ($r^s = -0.90$, n=45) and for *A. savannarum* ($r^s = -0.80$, n=33). When the maximum temperature was higher, both species had lower HR. In contrast, when the minimum temperature was low, the HR increased for *C. bairdii* and decreased for *A. savannarum*.

Study Limitations/Implications: We demonstrated the importance of considering quantifying the greatest number of variables when research is required on the selection and use of grassland bird habitat.

Findings/Conclusions: This study allowed us to increase our knowledge about the winter ecology of grassland birds and demonstrated that environmental variables such as annual rainfall and temperature influenced the habitat selection of *C. bairdii* and *A. savannarum*.

Keywords: Baird's sparrow, Grasshopper sparrow, wintering grassland birds.

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INTRODUCTION

The irregular distribution of rainfall and temperature rise in recent decades in different regions of the world are consequences of global climate change, which causes an alteration in the functionality of terrestrial ecosystems (IPCC, 2007; Böhning-Gaese *et al.*, 2008).

For the arid regions of North America, it has been modeled that changes and fluctuations in temperature and rainfall are deeper (Huang *et al.*, 2016), thus altering the habitat of plant species (Turnbull *et al.*, 2010) and animals, especially birds (Cohen *et al.*, 2020).

In this regard, the effect on the adaptive response of species (Zuckerberg *et al.*, 2009, Cady *et al.*, 2019) and avian communities (Jarzyna *et al.*, 2015) towards climate change has been documented. As well as the influence of variables such as rainfall in summer, on the abundance and temporal-spatial distribution of wintering grassland birds of the Chihuahuan Desert (Macías-Duarte *et al.*, 2018).

This group of birds in North America presents a strong population decline (−53%) in most of the species that represent it in the last 42 years (Rosenberg *et al.*, 2019). Therefore, to establish habitat conservation priorities at the continental scale (Berlanga *et al.*, 2010) it is necessary to generate and increase knowledge of the winter ecology of grassland birds.

In a similar way, this negative population trend of grassland birds is directly related to habitat loss and fragmentation throughout North America, which are more severe in the different wintering regions in the Chihuahuan Desert (Pool *et al.*, 2014).

This decrease is explained, among other causes, by changes in the net primary productivity of the grassland because of the irregular precipitation pattern of the region (Reichmann and Sala, 2014), by affecting the structure and coverage of the vegetation and in turn, the reproductive behavior of the birds (Conrey *et al.*, 2016) and their winter survival (Macías-Duarte *et al.*, 2017).

These effects translate into constant changes in the distribution and winter abundance of birds in the grasslands of northern Mexico (Panjabi *et al.*, 2013), as changing climatic conditions annually modify the structure and coverage of vegetation (comfort and protection against predation), the amount and distribution of food (seed biomass), modifying the territorial needs of birds so that they find and meet their vital requirements (Strasser *et al.*, 2019).

Under the assumption of the existence of a marked effect of climatic events on variables that affect the habitat characteristics of grassland birds; the objective of this study was to determine the effect of annual rainfall and winter temperature on some habitat characteristics (structure and coverage of winter vegetation and seed bank in the soil) and the size of the home range of two focal species (*Centronyx bairdii* and *Ammodramus savannarum*) of grassland birds in a grassland in northern Durango, Mexico, during four winter periods. This study allowed to increase the knowledge in the winter ecology of two species of birds with constant population decline and that this information can be useful in the development of strategies for the conservation of habitat and species.

MATERIALS AND METHODS

Study area

The study was implemented in a native grassland of 208 ha of extension, which is included within the cattle ranch "El Regalo" of the municipality of Hidalgo, north of the state of Durango, Mexico (Figure 1). It is located between the extreme geographical coordinates (26° 20'-11.24" N, 105° 10' 58.11" W and 26° 17' 5.98" N, 105° 9' 15.35" W), during the winter seasons 2015-2016, 2016-2017, 2017-2018, and 2018-2019.

Capture and processing of birds

The capture of the birds was made using bird mist nets on the line, black polyester model KTX of Avian Research Supplies, AFO, 36 mm mesh, 2.6 m high by 12 m long with four bags; eight people helped herding the birds towards the net (Panjabi and Beyer, 2010). For the capture, SEMARNAT granted the appropriate collection permits: SGPA/DGVS/104381/15, 10768/16, 011900/17 and 12947/18.

After the capture, on each of the birds a telemetry transmitter was placed on the back fastened with a harness made of elastic sewing band (Rappole and Tipton, 1991). The model of the transmitters was PicoPip 379 of LOTEK®, weighing 0.6 g, with a battery life of approximately 40 d (Panjabi and Beyer, 2010) and subsequently released at the same capture site.

Bird monitoring with transmitter

The birds were tracked daily by recording the coordinates of their location with a Garmin® GPS navigator model Etrex Vista. A database was generated with the locations

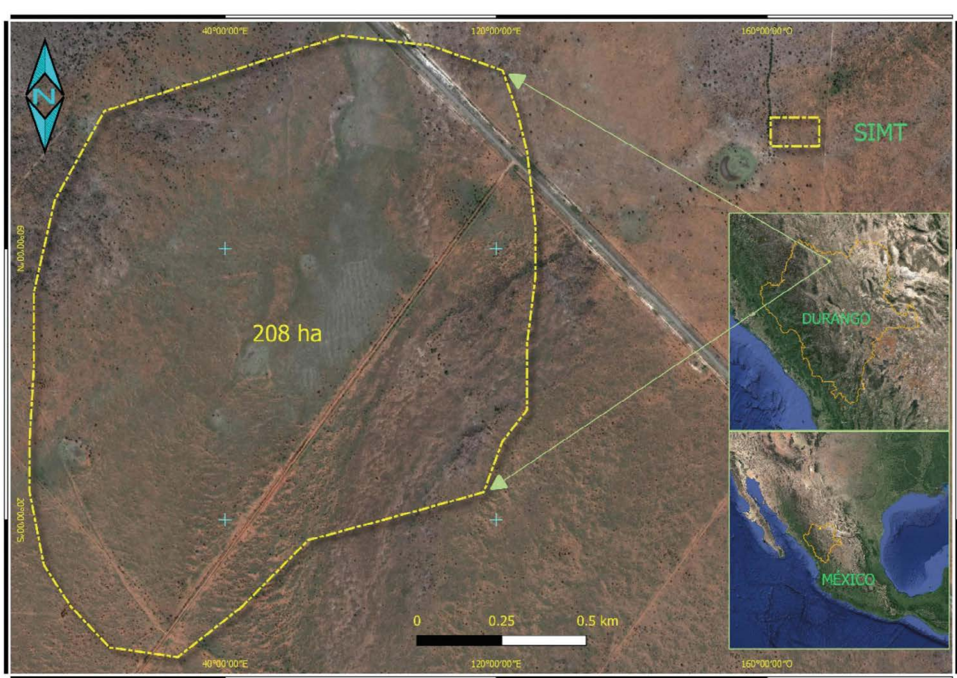

Figure 1. Location of the study site, "El Regalo" cattle ranch, at the municipality of Hidalgo, Durango.

by species and by individual, selecting those birds that had ≥30 detections, which is a fundamental requirement to estimate the home range of the individuals. Likewise, the location sites were also used to quantify the vegetation variables and to do the collection of soil samples to determine the seed biomass.

Estimation of the home range (HR)

The size of the home range (ha) was estimated using the fixed Kernel (K) method of 95% with least squares cross-validation (Seaman and Powell, 1996; Powell, 2000), using the software *adehabitatHR* version 3.3.1 (Calenge, 2006; R Core Team, 2018). To avoid overvaluation caused by occasional bird movements, those location points farther from the center of gravity in the cloud were removed prior to the estimate.

Sampling of vegetation (habitat) and seed biomass in the soil

The coverage percentage of herbs, shrubs, grassland, bare ground and other covers (dead vegetation and animal excrement) was visually estimated (Macias-Duarte *et al.*, 2018). The height of the vegetation was measured with a 1m Robel pole, marked every 2 cm (Robel *et al.*, 1970) in a circular area of 5m radius, around the location point of the birds, whose center was the central point of the geographical reference of the birds.

Seeds were collected at each point of location, using a metal ring of 8.8 cm in diameter; pressing it 0.5 cm deep into the soil and turning it half a turn to the right to recover the soil content (Desmond *et al.*, 2008). Subsequently, with the use of a metal spatula and a brush, the contents were placed on paper envelopes of 10×15 cm, identified with a label.

Seeds were separated from the rest of the soil content at the Laboratorio de Postgrado of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) under the Juarez University of the State of Durango (UJED). Seeds were separated through 1.0 and 0.5 mm mesh sieves, then observed under a 10×20 stereoscopic microscope (Leica®, model EZ4). The seed biomass per unit area (g m^{-2}) was quantified in a Sartorius® analytical balance, model CP224S (Desmond *et al.*, 2008).

Meteorological data collection

The data of annual rainfall (mm) of the years 2015-2019 and of minimum and maximum temperature of the winter months (December to February) of each period, were obtained from the meteorological station "Revolución" of the National Meteorological Service (SMN), of the Federal Government of Mexico. That weather station is near the study site, data was accessed through the National Water Commission (CONAGUA).

Statistical analysis

In this study, to relate the winter periods, the dependent variables (vegetation cover and height, seed biomass and home range) were grouped with the independent variables of annual rainfall (mm) ordered from lowest to highest amount of rain, and with the corresponding maximum and minimum winter temperature (°C) values. The Kruskal-Wallis non-parametric test was used ($p \leq 0.05$).

Additionally, to estimate if annual rainfall, minimum and maximum winter temperatures were correlated to the amount of seed biomass in the soil, a Spearman correlation analysis (r^s) was performed. The statistical analyses were performed with the NCSS® (Number Cruncher Statistical System – Hintze, 2001).

RESULTS AND DISCUSSION

In this research, during the four winter seasons (2015-2019) 78 birds were monitored with telemetry transmitter. Of those, 45 individuals corresponded to *Centronyx bairdii* (BAIS) and 33 to *Ammodramus savannarum* (GRSP) (Table 1).

Individuals of *Centronyx bairdii* selected sites with an average grass cover of 4.94%, grass height of 29.5 cm, shrub cover 3.79%, shrub height 42.53 cm, grass cover 75.18%, grass height 34.44 cm, bare ground 9.32% and other covers (dead vegetation and animal excrement) 6.75%.

Their average home range size was 0.80±0.11 ha and the amount of seed biomass in the soil was 2.70 g m^{-2}.

Individuals of *Ammodramus savannarum* used sites with grass cover 4.2%, grass height 29.4 cm, shrub cover 3.54%, shrub height 51.8 cm, grass cover 74.3%, grass height 32.4 cm, bare ground 9.1% and other covers (dead vegetation and animal excrement) 8.8%.

The size of the home range was 0.68 ha on average and the amount of seed biomass in the soil was 2.50 g m^{-2}.

Table 1. Number of birds studied by species and by winter season (year) in Durango, Mexico.

Winter season	*Centronyx bairdii*	*Ammodramus savannarum*
2015-2016	13	6
2016-2017	10	6
2017-2018	6	8
2018-2019	16	13

Table 2. Effect of annual rainfall on winter habitat characteristics, home range and seed biomass for *Centronyx bairdii* in a natural grassland in northern Durango, Mexico.

Variable	2015-2016	2017-2018	2018-2019	2016-2017
Annual rainfall (mm)	421.6	518.2	579.5	616.5
Herbs cover (%)	2.36 ± 0.8^{b}	3.70 ± 0.7^{b}	7.06 ± 1.0^{a}	6.63 ± 0.5^{a}
Herbs height (cm)	28.60 ± 3.2^{ab}	31.96 ± 3.1^{ab}	34.26 ± 1.5^{a}	23.60 ± 1.4^{b}
Shrubs cover (%)	6.33 ± 1.2^{a}	1.26 ± 0.2^{c}	2.63 ± 0.6^{bc}	4.93 ± 0.6^{ab}
Shrubs height (cm)	61.15 ± 10^{a}	30.83 ± 4.9^{b}	47.90 ± 10^{ab}	30.26 ± 1.8^{b}
Grass cover (%)	78.03 ± 3.2^{ab}	71.86 ± 1.7^{b}	71.26 ± 1.6^{b}	79.56 ± 1.1^{a}
Grass height (cm)	39.66 ± 2.5^{a}	38.26 ± 1.7^{a}	38.53 ± 2.6^{a}	21.3 ± 1.1^{b}
Bare ground (%)	7.63 ± 1.8^{b}	9.46 ± 0.8^{ab}	13.43 ± 1.3^{a}	6.76 ± 0.7^{b}
Other cover (%)	5.63 ± 1.0^{b}	13.7 ± 1.0^{a}	5.60 ± 0.7^{b}	2.1 ± 0.2^{c}
Home range (ha)	1.78^{a}	1.2^{a}	0.04^{b}	0.19^{b}
Soil seedbank (g/m^2)	0.99^{d}	1.69^{c}	4.79^{a}	3.35^{b}

Different letters among columns indicate significant difference (K-W, $p\leq0.05$).

Table 3. Effect of annual rainfall on overwintering habitat characteristics, home range and seed biomass for *Ammodramus savannarum* in a natural grassland in northern Durango, Mexico.

Variable	2015-2016	2017-2018	2018-2019	2016-2017
Annual rainfall (mm)	421.6	518.2	579.5	616.5
Herbs cover (%)	2.36 ± 0.8^{b}	3.70 ± 0.7^{b}	7.06 ± 1.0^{a}	6.63 ± 0.5^{a}
Herbs height (cm)	26.30 ± 3.4^{a}	27.60 ± 2.0^{a}	29.70 ± 1.8^{a}	21.90 ± 1.4^{a}
Shrubs cover (%)	4.86 ± 0.8^{a}	1.50 ± 0.2^{b}	3.60 ± 0.4^{a}	4.20 ± 0.5^{a}
Shrubs height (cm)	45.65 ± 5.0^{b}	52.30 ± 7.6^{b}	72.80 ± 6.0^{a}	36.56 ± 2.9^{b}
Grass cover (%)	82.76 ± 2.2^{a}	67.20 ± 2.3^{b}	61.40 ± 1.1^{b}	80.83 ± 1.7^{a}
Grass height (cm)	41.43 ± 2.9^{a}	36.83 ± 1.8^{a}	33.83 ± 1.1^{a}	17.46 ± 1.1^{b}
Bare ground (%)	6.63 ± 1.3^{bc}	15.00 ± 1.8^{a}	9.66 ± 0.5^{b}	5.33 ± 0.7^{c}
Other cover (%)	4.26 ± 1.0^{b}	14.4 ± 1.0^{a}	12.90 ± 0.7^{a}	3.63 ± 0.6^{b}
Home range (ha)	1.52^{a}	1.09^{b}	0.06^{c}	0.07^{c}
Soil seedbank (g/m^2)	1.33^{b}	3.38^{a}	1.76^{b}	3.54^{a}

Different letters among columns indicate significant difference (K-W, p≤0.05).

The results of the effect of minimum and maximum temperature during the winter months on habitat selection, home range (ha) and availability of seed biomass in the soil (g m^{-2}) are shown in Table 4 for *Centronyx bairdii* and in Table 5 for *Ammodramus savannarum*. Winters are compared within minimum temperatures (°C) (the coldest and the less cold) and maximum temperatures (the warmest and the less warm), for the other two winters there were no temperature differences between them.

For *Centronyx bairdii* (BAIS) overwintering home range (OHR) had a negative correlation (r^s) of −0.90 with the annual rainfall (AR), which indicates that the greater the amount of annual rainfall the size of the home range is smaller. There were other correlations, 0.94

Table 4. Effect of the minimum and maximum temperature during the winter months (December–February) on the characteristics of the winter habitat, home range and seed biomass for *Centronyx bairdii* in a grassland in northern Durango, Mexico.

Variable	Winter minimum (°C) temperature		Winter maximum (°C) temperature	
Average record	−10.3	−3.1	23.0	28.1
Herbs cover (%)	2.36 ± 0.8^{b}	7.06 ± 1.0^{a}	3.70 ± 0.7^{b}	7.06 ± 1.0^{a}
Herbs height (cm)	28.60 ± 3.2^{a}	34.26 ± 1.5^{a}	31.96 ± 3.1^{a}	34.26 ± 1.5^{a}
Shrubs cover (%)	6.33 ± 1.2^{a}	2.63 ± 0.6^{b}	1.26 ± 0.2^{a}	2.63 ± 0.6^{a}
Shrubs height (cm)	61.15 ± 10^{a}	47.90 ± 10^{b}	30.83 ± 4.9^{a}	47.90 ± 10^{a}
Grass cover (%)	78.03 ± 3.2^{a}	71.26 ± 1.6^{a}	71.86 ± 1.7^{a}	71.26 ± 1.6^{a}
Grass height (cm)	39.66 ± 2.5^{a}	38.53 ± 2.6^{a}	38.26 ± 1.7^{a}	38.53 ± 2.6^{a}
Bare ground (%)	7.63 ± 1.8^{b}	13.43 ± 1.3^{a}	9.46 ± 0.8^{a}	13.43 ± 1.3^{a}
Other cover (%)	5.63 ± 1.0^{a}	5.60 ± 0.7^{a}	13.7 ± 1.0^{a}	5.60 ± 0.7^{b}
Home range (ha)	1.78^{a}	0.04^{b}	1.2^{a}	0.04^{b}
Soil seedbank (g/m^2)	0.99^{b}	4.79^{a}	1.69^{b}	4.79^{a}

Different letters among columns and temperatures indicate significant difference (K-W, p≤0.05).

Table 5. Effect of the minimum and maximum temperature during the winter months (December-February) on the characteristics of winter habitat, home range and seed biomass for *Ammodramus savannarum* in a grassland in northern Durango, Mexico.

Variable	Winter minimum (°C) temperature		Winter maximum (°C) temperature	
Average record	−10.3	−3.1	23.0	28.1
Herbs cover (%)	1.46±0.1[b]	7.04±0.6[a]	1.90±0.3[b]	7.04±0.6[a]
Herbs height (cm)	26.30±3.4[a]	29.70±1.8[a]	27.60±2.0[a]	29.70±1.8[a]
Shrubs cover (%)	4.86±0.8[a]	3.60±0.4[a]	1.50±0.2[b]	3.60±0.4[a]
Shrubs height (cm)	45.65±5.0[b]	72.80±6.0[a]	52.30±7.6[b]	72.80±6.0[a]
Grass cover (%)	82.76±2.2[a]	61.40±1.1[b]	67.20±2.3[a]	61.40±1.1[a]
Grass height (cm)	41.43±2.9[a]	33.83±1.1[a]	36.83±1.8[a]	33.83±1.1[a]
Bare ground (%)	6.63±1.3[a]	9.66±0.5[a]	15.00±1.8[a]	9.66±0.5[b]
Other cover (%)	4.26±1.0[b]	12.90±0.7[a]	14.4±1.0[a]	12.90±0.7[a]
Home range (ha)	1.52[a]	0.06[b]	1.09[a]	0.06[b]
Soil seedbank (g/m^2)	1.33[a]	1.76[a]	3.38[a]	1.76[b]

Different letters among columns and temperatures indicate significant difference (K-W, $p \leq 0.05$).

with the minimum winter temperature (mWT), which suggests that this species requires larger territories when the winter months are colder; −0.80 with the maximum winter temperature (MWT), negative relationship that indicates that when the winter months are warmer the birds use smaller territories; and −0.86 with the amount of seed biomass, which explains that this species makes use of smaller spaces when it finds a greater amount of seeds ($p \leq 0.05$) (Figure 2).

For *Ammodramus savannarum* (GRSP) the OHR is also negatively and significantly related ($p \leq 0.05$) r^s of −0.80 with the AR; correlated −0.94 with the mWT; −0.80 with the MWT; and -0.40 with the amount of seed biomass.

Overall, there were no significant differences between the characteristics of the winter vegetation used by both sparrow species, except shrub height; where GRSP on average selected sites with greater height than BAIS (51.8 cm *vs.* 34.8 cm). In this regard, Macías-Duarte *et al.* (2017) in Janos, Chihuahua reported that GRSP used shrubs taller than BAIS (105 cm *vs.* 49.9 cm), regardless of the botanical composition of both sites.

The average overwintering home range (OHR) for the years of study was higher ($p \leq 0.05$) for BAIS (0.8 ha) than for GRSP (0.6 ha). In this regard, Pérez-Ordoñez (2019) in the grasslands of Marfa, Texas reported that the OHR of BAIS was 6.58 ha, and that of GRSP, 4.74 ha. Rechetelo *et al.* (2016) mentioned that the movement patterns of these sparrow species are irregular and changeable year after year. As well as the size of the overwintering home range, especially in species of granivorous birds called "extensive nomads", is due to the spatial and temporal variability of the distribution of seeds and rain. This also explains the behavior of that variable (AR) for both species, the greater the amount of rain, the lower the space requirement.

The size of the home range, the movements pattern and habitat use depend on the abundance, distribution, and availability of resources (Jenkins, 1981). During this study,

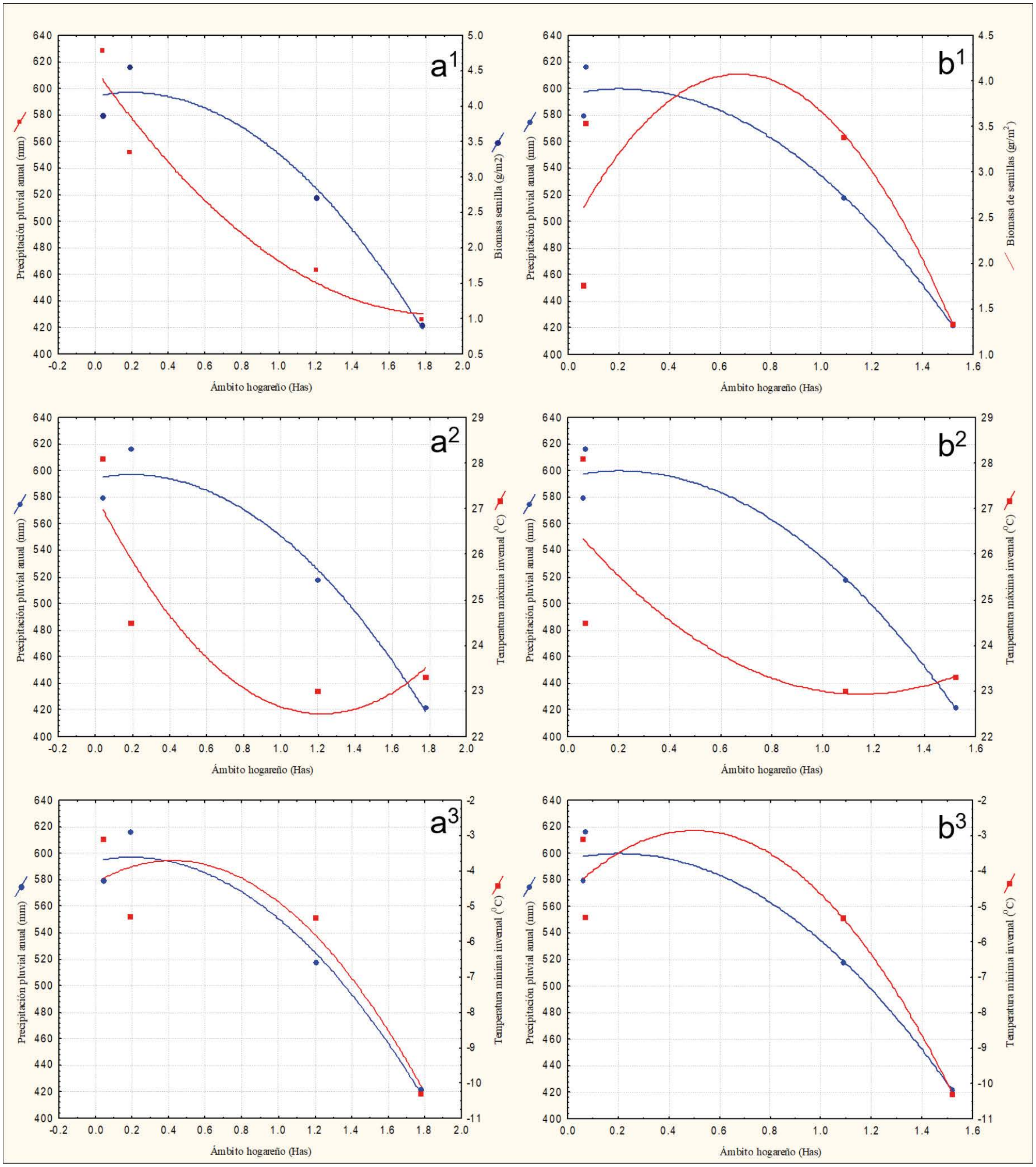

Figure 2. Correlations of the size of the overwintering home range of *Centronyx bairdii* (a) and *Ammodramus savannarum* (b) to annual rainfall, in regard to seed biomass (1) minimum winter temperature (2) and maximum winter temperature (3) in a grassland of northern Durango, Mexico. NOTE: (ha) is the correct SI unit to use for the overwintering home range (OHR), instead of (ha) in this figure.

both species used sites on average with the same amount ($p \leq 0.05$) of seed biomass (BAIS 2.7, and GRSP 2.5 g m^{-2}), data that differs with those quantified by Cabanillas (2016) who recorded 1.65 g m^{-2} for BAIS and 2.22 g m^{-2} for GRSP; and those of Salazar-Sánchez *et al.* (2021) who found other values in the same region (BAIS 1.69, GRSP 3.38 g m^{-2}). Studies indicate a trend of *A. savannarum* compared to *C. bairdii* to use sites that have more seeds in the soil, which is consistent with our data. However, the values of the number of seeds in the soil were much lower than those estimated by Méndez-González (2010) at sites used by grassland sparrows in New Mexico, USA with seed amount values of 4.0 and 14.0 g m^{-2}.

The effect of AR on the variables of land cover and vegetation structure for both species was not noticeable, the values coincide with those reported by Martínez *et al.* (2011) and Sierra *et al.* (2019); highlighting these obligate grassland birds selection sites with more than 60% of grass cover (USDA-NRCS, 1999).

In that sense, AR influenced the amount of seed biomass in the soil (g m^{-2}) and consequently on the size (ha) of the overwintering home range required, since rainfall is a key factor for the functionality of arid and semi-arid ecosystems; in addition, the composition and dynamics of vegetation depend on rainfall quantity and seasonality (Dudney *et al.*, 2017).

Rainfall affects the physiology of grasses (Connor and Hawkes, 2018). Dry years produce stress on plants, reduce their size and surface of their leaves, which reduces their photosynthetic capacity, causes hypoxia and nutritional deficiency (Steffens *et al.*, 2005). These alterations decrease growth, net primary productivity, and the ability of plants to produce seeds (Reichmann and Sala, 2014). Conversely, rainy years increase seed and biomass production in pastures while reducing the amount of herbs coverage (Dudney *et al.*, 2017). During this study, such an effect was observed only for seed production at sites used by birds. Seed availability influences the selection and space that birds require over the winter. Due to this, protection against predation, thermal coverage, and the availability of food (seeds), are the main elements that define the size of the territory that a species needs (Powell and Mitchell, 2012). Therefore, birds are excellent indicators of changes caused by severe environmental conditions such as droughts (Cady *et al.*, 2019) and extreme temperatures (Cohen *et al.*, 2020).

Similarly, the variation in the amount of rainfall accumulated annually at the study site affected the availability of necessary resources that birds require for wintering and the size of their territories. For example, birds required more space in years with less rainfall to meet their food needs; this behavior has also been recorded during the breeding season of grassland birds, where their reproductive success was greater in years preceded by greater rainfall (Zuckerberg *et al.*, 2018).

The effect of the minimum and maximum winter temperature (°C) on the size of the overwintering home range for both species was similar, birds use larger sites when the winter months are colder. However, *C. bairdii* preferred sites with greater cover and shrub height, without significant differences among the four years of study for the variables height and grass cover when combined the minimum and maximum temperatures were minor. Unlike *A. savannarum* which covered the requirement of thermal comfort in sites with greater grass cover.

In this regard, Cohen *et al.* (2020) indicated that bird species respond differently to extreme temperature events; small-sized species such as those in our study are more sensitive due to their low thermal inertia compared to larger birds, which have a faster adaptive response (Huey *et al.*, 2012). Low temperatures and poor vegetation cover during the winter limit the survival of both bird species (Perez-Ordoñez *et al.*, 2022).

CONCLUSIONS

There was evidence that during four winter seasons in a grassland in northern Durango, Mexico, annual rainfall, minimum and maximum temperature in the winter months determined habitat selection, the size of the home range and the amount of seed biomass in the soil, as a potential food for *Centronyx bairdii* and *Ammodramus savannarum*.

The selection of the winter habitat of both species was oriented towards sites with good vegetation cover, and low proportion of bare ground. A strong effect of rainfall was not observed on some specific characteristics such as grass cover for *C. bairdii*, but for *A. savannarum*, which used sites with a lower proportion of this type of cover.

The overwintering home environment of the sparrow species studied had a negative correlation with rainfall. Birds tended to use smaller territories in the years of higher annual precipitation, the amount of precipitation also caused that wintering sites with a greater number of seeds in the soil were selected. This phenomenon was more marked in *C. bairdii* than in *A. savannarum*; however, for both species these values were statistically significant.

The effect of the minimum and maximum winter temperature on the home range of the studied species was evident. Both reacted to extreme cold events using larger areas, even tough did not contain much potential food (seeds). However, in the face of warmer events (higher maximum temperature) in the winter, only *C. bairdii* used smaller spaces, but with more seeds; unlike *A. savannarum* that although also used smaller spaces, those contained smaller amounts of seeds.

Although annual rainfall had the most prominent effects to provide the best survival conditions for birds, it is recognized that the selection and use of territories by the sparrow species studied may have been due to a synergistic effect of the environmental variables studied.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Yield and oil content of castor oil plant (*Ricinus communis* L.) accessions grown with fertigation in a semiarid zone

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ABSTRACT

Objective: To evaluate seed, dry matter, and oil content yield in five accessions of castor oil plant (*Ricinus communis* L.) in the Altiplano Potosino Zacatecano high plateau regionof Mexico, grown with a fertigation system.

Design/Methodology/Approach: Castor was established under a fertigation system on agricultural land. Three levels of fertilization were tested. A completely random experimental design was used, with a 5×4 factorial arrangement (accessions×fertilization levels) and three repetitions, for a total of 60 experimental units.

Results: The following accessions showed an outstandingly favorable response to fertilization: ZACS2C1 (Orito Zac) which had the greater seed yield (735 g) and oil content (%) (50.30±5.23); and SLPS11C1 which had the greater weight per 100 seeds (g) (56.77±2.35) and dry matter (1600 g). The best fertilization level for the above mentioned accessions was obtained with the low nutrient solution, while other accessions showed a variable and inverse response to the fertilization level.

Limitations/Implications of the study: The study did not imply limitations.

Results/Conclusions: The castor oil plant accession from El Orito showed a favorable response to fertilization levels and achieved a higher yield (number of seeds and their oil content), even in the absence of fertilization; consequently, it is a promising accession for agro-industrial crops use.

Keywords: accessions, fertigation, nutrition, productivity, *Ricinus communis*.

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INTRODUCTION

The castor oil plant (*Ricinus communis* L.) species can be found from sea level up to 2,500 m.a.s.l. (Naik, 2018). Its water requirement ranges from 75 to 1,178 mm, depending on the soil type, the irrigation method, and its location (Ramanjaneyulu *et al.*, 2013). It is basically a long-day plant, but it can be adapted to a very wide range of daylight, with lower yield and a wide range of photoperiods (Naik, 2018). Its seed produces an oil that is used for a wide range of industrial purposes (Kallamadi *et al.*, 2015). The main differences between the diverse varieties lay on the color and shape of their stems, leaves,

Image by Alejandro Sánchez

and seeds; they grow as perennial plants in tropical and subtropical regions (frequently growing above 7 m tall) and as annual plants in temperate climates. The main producers of castor oil plant seeds worldwide are: India (1.2 million t), Mozambique (85,000 t), China (27,000 t), Brazil (14,000 t), and Ethiopia (11,000 t) (FAOSTAT, 2018; the figures are approximations). According to SIAP (2019), the main castor oil plant producers in Mexico are: Sonora (12,194 t/11,553 ha), Sinaloa (1,620 t/1,000 ha), and Nayarit (1,695 t/660 ha), with estimated production values of $83.8, $5.6, and $17.5 million Mexican pesos, respectively. The demand for castor oil in Mexico has increased during recent years (Trademap, 2019); various industries use it as an additive or raw material for their products, including the automotive, cosmetics, pharmaceutical, lubricant, paint, and ink industries. However, the low supply offered by the Mexican industry has forced the industrial sector to import crude or refined oil from India, Spain, Brazil, Germany, and the United States, among others. Overall, the production of castor oil plants includes wild, semi-wild, intermediate, and hybrid populations (Velasco *et al.*, 2015). Insufficient P and K levels slow down the initial growth of the plant and may lead to a considerable productivity reduction (Severino, 2006). Additionally, the growth and production of castor oil plant requires high N doses; a deficiency of this element leads to few leaves and small-sized plants (Araujo *et al.*, 2009). On general terms, reports mention 860 kg/ha yields (Rivera and Hernández 2016). Therefore, this work tested six promising samples of genotypes of castor oil plant (*Ricinus communis* L.) with various fertilization levels, in order to determine their viability as a systematic crop.

MATERIALS AND METHODS

Plant material and location of the experiment

The castor oil plant (*Ricinus communis* L.) accessions belong to a germplasm bank obtained at the influence area of the CP Campus San Luis (Table 1). The experimental castor oil plant cultivation was established at 101° 22' 33" N and 22° 43' 51" W, at an altitude of 2,100 m.a.s.l., in the Vicente Guerrero town, Salinas de Hidalgo municipality, San Luis Potosí. According to the data obtained by the COLPOS-SLP weather station, the rainfall accumulated during this research amounted to 467 mm and the average temperature was 15.5 °C.

Table 1. Origin of the *Ricinus communis* L. accessions.

Identification key	Accession Name	Latitude (N)	Length (O)	Altitude (msnm)
ZACS2C1	El Orito, Zacatecas	22° 45' 07.8"	102° 35' 36.4"	2,399
JALS2C1	Encarnación Díaz, Jalisco	21° 31' 12.8"	102° 14' 47.1"	1,811
SLPS11C1	Salinas San Luis Potosí	22° 37' 28.9"	101° 42' 52.3"	2,083
SLPS1C1	Ranchería de Guadalupe, San Luis Potosí	22° 19' 42.0"	101° 12' 04.6"	1,861
SLP*	San Luis Potos	-	-	-

* Castor oil plant seed with varietal origin, collected in three Mexican states.

Preparation of plant material for cultivation

To grow the seedlings, the seeds were put 40 days in 2.5-L black bags with a substrate made of a mixture of sand, tezontle, and compost (in equal parts), using a 4-L h^{-1} localized dripping irrigation system. During the said period, most plants had 5 leaves. Afterwards, they were transplanted into a plot where the land had been previously turned over (using a chisel plough and a harrow) and the soil had been levelled. Sixty plants were distributed in a 240 m^2 plot, 1.5 m apart from each other. Likewise, to prevent the border effect, a row of plants was sown on the border of the study accessions.

Soil fertigation system

Four 1,000-L water tanks were used for the fertilizer doses and a 4-L/h dripper was used per plant. The Penman-Monteith formula was used to estimate irrigation, based on the data obtained from the COLPOS-SLP weather station, located 2 km away from the study site. Water application was controlled with three control drippers. In the final stages of the evaluation, the soil was irrigated twice a day, in 30- and 40-min application periods.

Fertilization levels

The following NPK (ppm) fertilization levels were applied (Table 2): without fertilization (control), low (140:50:150), medium (210:80:250), and high (280:110:350). The combinations were prepared in independent containers. Each nutrient solution was complemented with secondary nutrients and micronutrients (Fe, Zn, Cu, and B). Additionally, monoammonium phosphate and monopotassium phosphate were used to prepare the nutrient solution, during the growth and reproductive stages, respectively.

Study variables and experimental design

The following variables were evaluated during the research: seed yield (g $plant^{-1}$), dry matter production (g $plant^{-1}$), and oil content. The latter was determined according to the methodology described by Loreto *et al.* (2012), using a Soxtec oil

Table 2. Nutrient solution used in the soil (g/1,000 liters of water) to grow castor oil plant.

Nutrients	Fertilization levels		
	Low level	Medium level	High level
KNO_3	487.50	600.00	650.0
$Ca(NO_3)_2$	412.50	562.50	550.0
H_6NO_4P *	187.50	232.50	250.0
KH_2PO_4**	187.50	232.50	250.0
$MgSO_4$	90.00	90.00	120.0
$C_{18}H_{16}FeN_2NaO_6$	8.20	8.20	11.0
$CuSO_4$	0.60	0.60	0.8
$ZnSO_4$	0.52	0.52	0.7
H_3BO_3	1.35	1.35	1.8

Note: * Monoammonium phosphate used during the growth stage. ** Monopotassium phosphate used during the fruiting stage.

and fat extraction system (USA). The weight of 100 seeds was determined using an Ohaus analytical balance (Ref. Explorer Pro, with a ± 0,0001 g accuracy), according to Makkar *et al.* (1998). A completely random experimental design was used, with a 5×4 factorial arrangement (accessions×fertilization levels) and three repetitions, for a total of 60 experimental units.

RESULTS AND DISCUSSION

Castor oil plant seed yield

Overall, a high fertilization level resulted in a greater seed yield (Figure 1). The highest results were obtained with accession ZACS2_C1 (753 g plant^{-1}), followed by SLP11_C11 (644 g plant^{-1}). These yields are lower than the results reported by Solís *et al.* (2010) for 10 varieties improved by INIFAP, obtained from Chiapas, State of Mexico, and Michoacán, with a 0.297-1.106 kg plant^{-1} weight.

Clearly, the SLPS1_1 accession showed a better yield when medium and low fertilization levels were applied. This means that this genotype might have low fertilization requirements, a possibility that requires further research. However, accession AZCS2_C1 showed remarkable responses to the fertilizers applied and might therefore be a genotype with high production requirements. The lower production may have been caused by differences in weather conditions between the regions where the crop was grown and the previous genetic selection work that was carried out. Consequently, the next activity should be a cost-benefit economic analysis, in order to determine if investing in seed yield is convenient.

Dry matter (g plant^{-1})

The greatest amount of dry matter was produced by accession SLPS11_C1 with all fertilization levels; the amount was significant between accessions and fertilization levels.

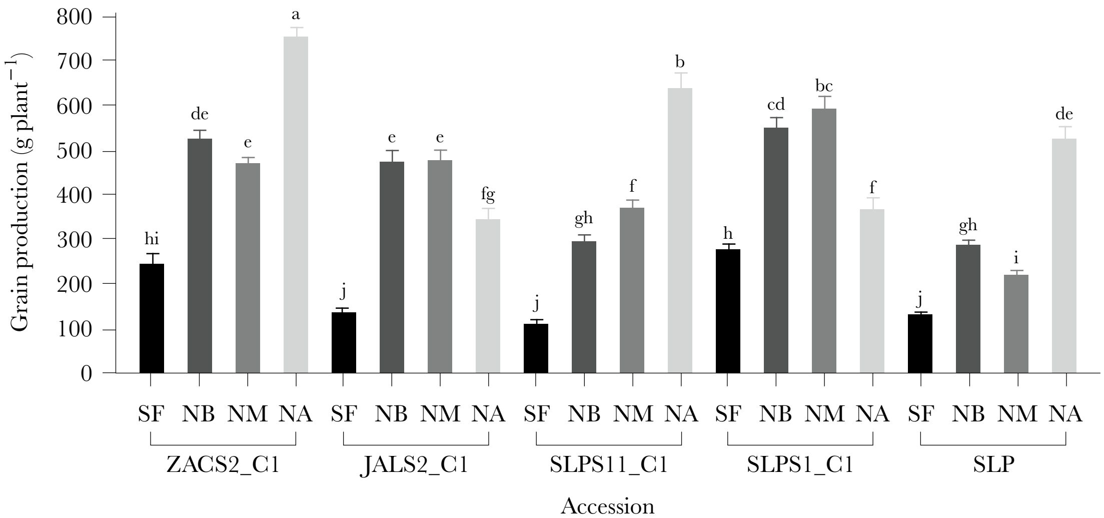

Figure 1. Seed yield of *R. communis* L. accessions per fertilization level.
SF: without fertilizer, NB: low level, NM: medium level, NA: high level.

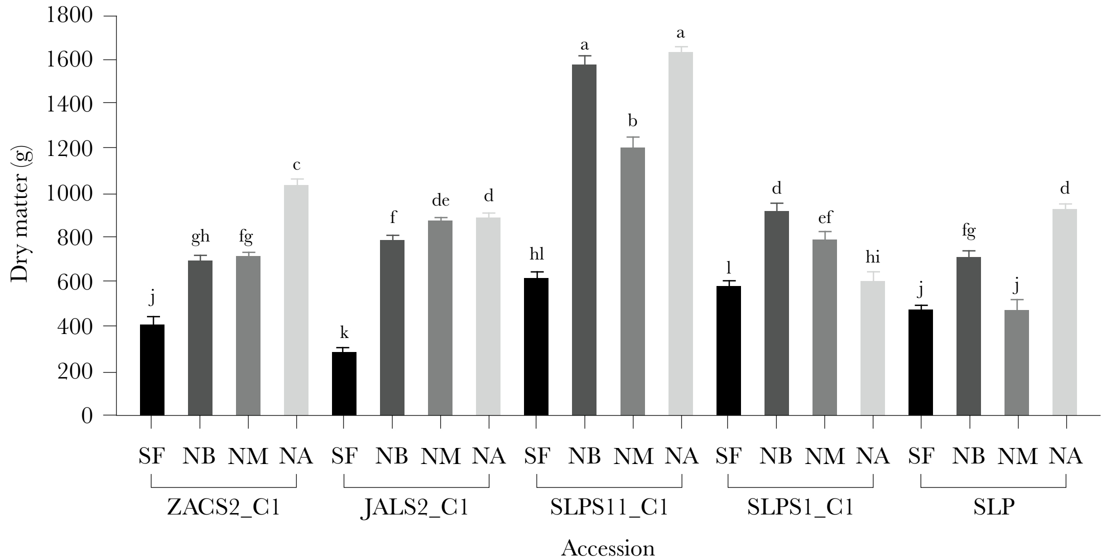

Figure 2. Dry matter behavior (g plant^{-1}) of castor oil plant (*R. communis* L.) accessions about fertilization levels. SF: without fertilizer, NB: low level, NM: medium level, NA: high level.

In accessions ZACS2_C1 and JALS2_C1, dry matter increases in relation to the increase of fertilization levels. Accession SLPS1_C1 had an inverse trend about fertilization levels. Accesion SLP had a highly illogical trend about the fertilization level. No relation was found between seed yield and the accession that produced more dry matter. A higher dry matter yield would provide the producers with an additional economic benefit, as the output could be used as fodder and/or soil conditioner. The average plant height ranges from 156 cm (accession from Ranchería de Guadalupe) to 249 cm (accession from Salinas); similar heights have been reported in other studies carried out in similar environments.

Accession JALS2_C1 showed low response to the fertilizers used.

Weight of one-hundred seeds (g)

Seeds from accession SPLS11_C1 grown without fertilizer achieved the highest weight (56.77 g±2.35), with a diminishing trend at greater fertilization (Table 3). The seed weight of accession SLP showed a homogeneous stability, probably due to the selection

Table 3. Weight of one-hundred seeds (g) of the *Ricinus communis* L. accessions.

Accession	Without Fertilizer	Low level	Medium level	High level
ZACS2_C1	11.50±1.20^{f}	12.70±0.70^{f}	12.83±0.65^{f}	12.40±0.50^{f}
JALS2_C1	30.47±1.05^{e}	33.07±1.55^{e}	31.0±1.30^{e}	32.20±1.80^{e}
SLPS11_C1	56.77±2.35^{a}	55.07±0.75^{ab}	46.33±1.25^{c}	40.7±2.10^{d}
SLPS1_C1	11.37±1.15^{f}	11.73±0.55^{f}	10.60±0.4^{f}	11.0±0.70^{f}
SLP	51.20±1.81^{b}	52.07±1.55^{b}	54.30±1.9^{ab}	51.37±1.15^{b}

The results are set forth as the mean of three replications±standard deviation. Different letters in the same column show significative differences ($p<0.05$) in Tukey's test.

process it has been subjected to and which has allowed it to retain uniform characteristics; regrettably, it produces little dry matter and only responds to high fertilization levels. The differences between accessions are fundamentally nutrition-related and/or the result of the plant's intrinsic conditions (particularly, its genetic composition). The seed weight of the accessions is higher than the weight reported by Vasco *et al.* (2018) for a crop under controlled conditions (54.23 g). Perdomo *et al.* (2013) reported very low values (13.2-40 g) in accessions from the state of Queretaro. According to Beltrão *et al.* (2001), the weight of one-hundred castor oil plant seeds ranges from 10 to 100 g. The mean in dwarf-sized cultivars is 30 g and, in medium-sized cultivars, it ranges from 45 to 75 g, under cultivation conditions. Accessions with low-weight seeds produce more seeds per plant; this would seem to be one of the species' survival strategies.

Oil content in the *R. communis* L. accessions

The oil content of the various castor oil plant accessions with the different fertilization levels fluctuated between 22.75 and 50.30% (Table 4); accession ZACS2_C1 recorded the highest content (50.30% with a high fertilization level). In this regard, Vasco *et al.* (2017) reported similar high results for other wild regional accessions: ZACS2C1, JALSC1, and SLP11C1, with 48.84, 50.14, and 51.04% values, respectively. Therefore, a new formulation of the fertilization levels should be discussed. This work confirmed that more oil was obtained from the accession with the highest number of seeds and the lowest seed weight.

CONCLUSIONS

The castor oil plant accession from El Orito showed a favorable response to fertilization levels and achieved a higher yield (number of seeds and their oil content), even in the absence of fertilization; consequently, it is a promising accession for agro-industrial use crops. A full use would require the use of SLPS11_C1 which has an outstanding seed weight and dry matter volume per plant.

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Table 4. Oil content with soil fertilization levels of *R. communis* L. accessions.

Accession	Without Fertilizer	Low level	Medium level	High level
ZACS2_C1	35.60 ± 7.92^{abcd}	41.00 ± 5.66^{abcd}	42.35 ± 1.63^{abc}	50.30 ± 5.23^{a}
JALS2_C1	34.65 ± 2.19^{abcd}	36.15 ± 4.88^{abcd}	33.45 ± 1.48^{abcd}	32.75 ± 7.85^{abcd}
SLPS11_C1	31.35 ± 0.78^{abcd}	22.75 ± 0.49^{d}	36.30 ± 1.13^{abcd}	34.05 ± 4.60^{abcd}
SLPS1_C1	32.25 ± 6.01^{abcd}	39.10 ± 2.12^{abcd}	26.55 ± 7.57^{cd}	38.75 ± 1.63^{abcd}
SLP	41.20 ± 7.64^{abcd}	48.00 ± 3.82^{ab}	24.95 ± 4.03^{cd}	45.60 ± 1.84^{ab}

The results are set forth as the mean of three replications±standard deviation. Different letters in the same column show significative differences ($p<0.05$) in Tukey's test.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Buffer capacity as a method to estimate the dose of liming in acid soils

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ABSTRACT
Objective: The study proposes using the dolomite buffer capacity ($BC_{Dolomite}$) as a method of estimating the lime requirement in soils that agronomically require a pH increase.
Design/methodology/approach: In six soil samples with different levels of response to the application of dolomite, the organic matter (OM), the content of sand, silt and clay, pH, K, Ca, Mg and the potential acidity (Al+H) were determined. Assuming an apparent density of 1000 kg/m^3 and a volume per hectare of 2000 m^3, the soils were placed in polyethylene containers and treated with dolomite in doses equivalent to 0, 750, 1500, 2250 and 3000 kg/ha, establishing the $BC_{Dolomite}$ as the inverse of the slope resulting from the relationship between the pH and the dose of dolomite.
Results: In soils with low response to the dolomite, the content of clay and OM was 280 and 26 g/kg, respectively, and in high response soils, the content of clay and OM was 240 and 47 g/kg, respectively. In all cases, a simple and direct linear relationship was observed ($R^2>0.85$; $p<0.05$) in the relationship between pH and dose of dolomite.
Limitations on study/implications: The results obtained under controlled conditions show that $BC_{Dolomite}$ constitutes a viable method to estimate the lime requirement.
Findings/conclusions: The $BC_{Dolomite}$ showed sensitivity to the complexity of the clay fraction and to the organic matter in the soil, for which the dolomite requirement is equal to the product of the desired pH increase and the $BC_{Dolomite}$.

Keywords: hydrogen, aluminum, dolomite, liming.

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INTRODUCTION
Some factors that cause acidification of soils are biological activity (plants and microorganisms), base washing (Ca, Mg, K) from the soil, decomposition of organic matter, and intensive production of crops (banana, oil palm, cacao, sugarcane), where the absorption of crops derives in an exhaustion of the soil bases and an increase in the saturation of aluminum and hydrogen (Agegnehu *et al.*, 2021; Goulding, 2016); and the degree of acidification, within a period of time, depends on the buffer capacity of the soil.

Image by Ioannis Karathanasis at Pixabay

The buffer capacity is defined as the soil's resistance to change pH in the presence of the addition of an acid or a base, and the buffer capacity depends on the amount and type of clay (silica, non-silica, fixed load and variable charge particles) present in the soil and on the content and degree of stabilization of organic matter (Sanchez, 2019). For this reason, sandy soils present a greater resistance to acidification.

In acid soils, the efficiency of fertilization (available nutrient/applied nutrient) and the efficiency of nutrient absorption (absorbed nutrient/available nutrient) decrease (Baligar *et al.*, 2001), which is explained knowing that the soil pH has a relationship with the precipitation, solubilization, immobilization, mineralization, adsorption, and desorption of essential elements, bioavailability of toxic elements, and reduction of biomass of functional roots (Baligar *et al.*, 2001; Neina, 2019), conditions which together limit crop production.

To exceed the agronomic limitations associated to the acidity of soils, they are treated with products that contribute calcium, magnesium or both (Ca and Mg), capable of reducing the acidity and increasing the pH of soils to a specific degree, depending on the neutralization value of the product (type of product and purity), the relative efficiency (particle diameter of the product), and the buffer capacity of the soil (Chimdi *et al.*, 2012). However, although the technical foundations that determine the effectiveness of liming products are known, the classic methods to establish the dose of lime do not consider the level of pH that is intended to be increased in the soil, since they are mostly based on the concentration of exchangeable aluminum or the degree of saturation of bases (Teixeira *et al.*, 2020).

Defining the dose of the liming product to reach an agronomically adequate pH in the soil is determinant to ensure a correct physical, chemical and biological functioning of the soil, which favors the growth, development and production of crops. With this background, this study proposes the use of the buffer capacity of the soil with double calcium-magnesium carbonate ($BC_{Dolomite}$) as a fast method to estimate the liming dose and to correct the acidity of the soils.

MATERIALS AND METHODS

Soil samples and physicochemical characterization

In the locality of Quevedo (Province of Los Ríos – Ecuador), having as criterion for soil sample selection the historical effect of liming on pH (low, medium and high response), six soil samples were collected classified within the Inceptisol order with a source of Andean influence (SIGTIERRAS, Sistema Nacional de Información y Gestión de Tierras Rurales e Infraestructura Tecnológica), 2017). The samples were identified as Soil A, Soil B, Soil C, Soil D, Soil E and Soil F. The samples Soil A and B are of low response, the samples Soil C, D and E of medium response, and the sample Soil F of high response. According to the classification system of ecosystems in Ecuador (Ministry of the Environment, MAE (*Ministerio del Ambiente del Ecuador*), 2013), the locality of Quevedo is found in a seasonal evergreen forest ecosystem characterized by a mean annual temperature of 25.5 °C and an annual precipitation of 1650 mm.

The soil samples were subject to a physical and chemical characterization, following procedures accepted by Ecuador's Society of Soil Science and described by Rodríguez and

Rodríguez (2015). In each soil sample organic matter was determined with the Walkley Black method; the sand, loam and clay content with the hydrometer method; the pH in water $\left(pH_{H_2O}\right)$ with the potentiometric method using a weight:volume ratio of 1:2.5; the exchangeable K, Ca and Mg, with the Olsen – Modified method; the potential acidity (Al+H) with the KCl 1N method and titration with NaOH 0,01N. Likewise, through a particle-size analysis and titration, the dolomite used in this study was determined to have a relative efficiency and neutralization value of 100 and 87%, respectively.

Buffer capacity

Based on the determination methods of the buffer capacity and the estimation of lime requirements described by Follett and Follett (1983), 10 polyethylene containers with screw tops were prepared for each soil sample, placing in each container 50 g of soil (dried at room temperature and sieved at 2 mm) and treated by duplicate with dolomite in doses equivalent to 0, 750, 1500, 2250 and 3000 kg/ha, assuming a volume per hectare of 2000 m^3 and an apparent density of 1000 kg/m^3. Then, the pH_{H_2O} was determined after incubation for 24 hours, at constant temperature (40 °C) and moisture (40% p/p). The $BC_{Dolomite}$ was determined by the inverse of the resulting slope when evaluating the variation of pH in function of the dose of dolomite added to the soil (Ng *et al.*, 2022).

Data analysis

The physical and chemical characterization of the soil samples is summarized presenting the arithmetic average and the standard deviation (Tables 1 and 2). The content of organic matter, loam, sand and clay, was determined immediately after the initial preparation of the soil samples (drying and sieving) (Table 1), while the pH_{H_2O}, content of K, Ca and Mg, and potential acidity (Al+H) were determined in the soil samples with and without dolomite treatment in doses equivalent to 1500 kg/ha (Table 2), and the statistical differences were established through a t-test for paired samples.

After the incubation period of the soil samples, the buffer capacity was evaluated through a regression analysis (Figure 1), relating the pH of each soil sample in function of the dose of dolomite (0, 750, 1500, 2250 and 3000 kg/ha). The following regression equation was obtained: , where: pH of the soil exposed to a specific dose of dolomite, slope associated to the dose of dolomite, intercept. In the model, the coefficient of determination (R^2) and the statistical significance (p-value) were determined.

RESULTS AND DISCUSSION

The results evidence that a slight change in pH (low response) of the soils facing the addition of dolomite cannot always be explained by a high content of clay, since the soil with a high response (Soil F) has a clay content (240 g/kg) similar to soils of low response (Soil A, Soil B) (average clay 280 g/kg), while the soils of medium response (Soil C, Soil D, Soil E) have an average content of clay (180 g/kg) lower than the soil with a high response (Soil F) (240 g/kg). The soils that have a medium response of pH change in the presence of the addition of dolomite, specifically soils C and D, have a low content of clay but a high content of organic matter (both soils with more than 6% of organic matter) (Table 1).

Table 1. Content of organic matter (OM), sand, lime and clay, and textural class of six soils belonging to the Inceptisol order from the locality of Quevedo, Los Ríos – Ecuador. SD=standard deviation.

Soil sample	**OM**	**Sand**	**Silt**	**Clay**	**Soil texture**
	g kg^{-1}				
Soil A	30	300	420	280	Clay loam
Soil B	22	360	360	280	Clay loam
Soil C	61	520	320	160	Loam
Soil D	66	540	320	140	Sandy loam
Soil E	17	460	300	240	Loam
Soil F	47	460	300	240	Loam
Mean	41	440	337	223	
SD	21	93	46	60	

The magnitude of pH change varies in the different soils facing the same dose of liming, evidencing that the soils differ in their resistance to change pH depending on the content of organic matter, type and amount of clay (Uthida and Hue, 2000); thus, in soil samples with similar pH in water (≈4,30) and $CaCl_2$ (≈3,75), differences of 11% were observed in the clay content (Sanaullah and Shamsuddin, 2010), and when evaluating the effect of the dose of dolomite in an Ultisol and an Inceptisol, an increase in the pH of 0.14 and 0.20 units/ton, respectively, was observed (Lukman and Yudha, 2020).

The solid reactive fraction (particles with colloidal characteristics) of the soils can be of permanent charge (vermiculite, illite, montmorillonite) or of variable charge (caolinite, allophane, ferrihydrite), reason why the clays can transfer H^+ to the medium from deprotonation, depending on whether the medium is acid or alkaline (Jeon and Nam, 2019). Therefore, the soils exhibit a variable resistance to changing pH, which explains why the soils with dominion of clays with permanent charge have a high response in the presence of the lime addition, contrary to what happens in soils with dominion of clays with variable charge.

The addition of 1500 kg/ha of dolomite caused a significant increase of pH of 0.43 tenths, with the pH of soil changing on average from 5.89 to 6.23, while no significant changes were observed at the level of exchangeable Ca and Mg of the soil since dolomite is a correction that contributes exchangeable Ca and Mg, the same that a change at the level of potential acidity (Al+H) was not observed; however, the ECEC increased significantly by 1.05 mEq/100g, with ECEC going on average from 9.66 to 10.71 mEq/100g (Table 2).

In acidic soils, predominant in tropical regions (high precipitation and temperature) and intense agricultural activity, exhaustion of the bases in the soil (K, Ca, Mg) and high saturation of Al^+ and H^+ (>50 %) are frequently observed (Jakovljević *et al.*, 2005; León-Moreno *et al.*, 2019), which is why liming is recurrent. Ca^{2+} or Mg^{2+} (from liming) concentrate in the soil and generate a mass effect that displaces the linked Al^+ (saturation of Al^+) to the clays (Amberger, 2006; Cunha *et al.*, 2018), which suggests a higher probability of hydrolysis of Al^{3+} and a higher resistance of the soil to increasing the pH, because the hydrolysis of Al^+ generates H^+ ions.

Table 2. Values of pH, exchange bases (K, Ca, Mg), potential acidity (Al+H), and effective cation exchange capacity (ECEC) of six soils, with and without dolomite treatment (calcium-magnesium carbonate), belonging to the Inceptisol order from the locality of Quevedo, Los Ríos – Ecuador. SD=standard deviation.

Soil sample	**pH**	**K**	**Ca**	**Mg**	**Al+H**	**ECEC**
		mEq 100g^{-1}				
	Dolomite dose=0 kg ha^{-1}					
Soil A	6,62	0,21	9,40	2,64	0,21	12,46
Soil B	6,30	0,19	8,44	1,99	0,25	10,87
Soil C	5,95	0,44	7,41	1,05	0,26	9,16
Soil D	5,35	0,48	6,00	0,96	0,38	7,82
Soil E	5,92	0,21	7,01	1,73	0,37	9,32
Soil F	5,20	0,84	6,32	0,92	0,26	8,34
Mean	5,89	0,40	7,43	1,55	0,29	9,66
SD	0,54	0,25	1,29	0,69	0,07	1,72
	Dolomite dose=1500 kg ha^{-1}					
Soil A	6,81	0,22	11,67	2,77	0,20	14,86
Soil B	6,59	0,18	8,45	2,05	0,27	10,95
Soil C	6,27	0,43	7,85	1,27	0,29	9,84
Soil D	5,51	0,49	6,11	0,99	0,42	8,01
Soil E	6,38	0,32	7,20	3,38	0,32	11,22
Soil F	5,83	0,88	6,97	1,23	0,28	9,36
Mean	6,23	0,42	8,04	1,95	0,30	10,71
SD	0,48	0,25	1,95	0,96	0,07	2,34
t-test (p-value)	0,0052	0,2361	0,1365	0,1755	0,5646	0,0408

Organic soils, or mineral soils with high contents of organic matter, exhibit a high resistance to changing pH, because in an acidic medium the carboxyl groups of the organic matter of the soil would adsorb H^+, and in an alkaline medium they would transfer H^+, aspects that agree with the findings by Chi *et al.* (2017), when they reported an increase in the buffer capacity of pH of an Ultisol by adding biochar derived from plant residues to the soil. Thus, taking into account the complexity of the interaction between organic matter and chemical elements, it is possible to explain that organic soils generally have a low response to liming compared to mineral soils, which are rich in clays with permanent charge.

Dolomite $\left[CaMg\left(CO_3\right)_2\right]$ or calcite $\left(CaCO_3\right)$, when entering into contact with the soil moisture, have the capacity of increasing the soil's pH when Ca^{2+}, Mg^{2+} are liberated, and OH^- is produced; thus, Ca^{2+}, Mg^{2+} are exchanged with Al^{3+}, H^+ is linked electrostatically with the solid fraction of the soil, while OH^- ions react with Al^{3+} and with H^+ to form $Al(OH)_3$ and H_2O, respectively. This explains why in barley fields (*Hordeum vulgare* L.) of the central high plateau of Ethiopia, a linear increase of the pH (5.20 to 5.90) is observed at increasing doses (0 to 220 g/m^2) of calcium carbonate, and the Al^{3+} decreases exponentially (1.35 to 0.15 mEq/100g) (Desalegn *et al.*, 2017). In addition, the studies reveal that there is not a stoichiometric relationship between the liming dose and the increase of pH observed.

The Ca^{2+} and Mg^{2+} ions contained in the dolomite increase the exchangeable calcium and magnesium in the soil, although the magnitude of increase of exchangeable calcium or magnesium depends on the quality (neutralization value and relative efficiency) and liming dose (Sanchez, 2019), because as a whole they control the ionic force generated in the soil solution and therefore the capacity of Ca^{2+} and Mg^{2+} of garnering the exchange sites when Al^{3+} and H^{+} are displaced. Thus, in a 10-year study carried out with a Typic Palexerult soil, an increase of exchangeable calcium and magnesium was observed after the incorporation of 10900 kg/ha of dolomite in the first 20 cm of soil and, therefore, an increase of the effective cation exchange capacity (ECEC) and a reduction of the exchangeable aluminum (Al^{3+}) (Olego *et al.*, 2021).

When evaluating the pH variations of the soil against the dose of dolomite, a positive (direct) correlation was observed in every case, which adjusts to a simple linear model (R^2>0.85; $p<0.05$). In addition, the lower response of the soil to liming was seen to correspond with a lower value of the slope of the linear model, and a higher response of the soil corresponds to the higher value of the slope. It was found that in the soils with low (Figures 1a and 1b) and high (Figure 1f) response, the slope was 0.00015 and 0.00049, respectively.

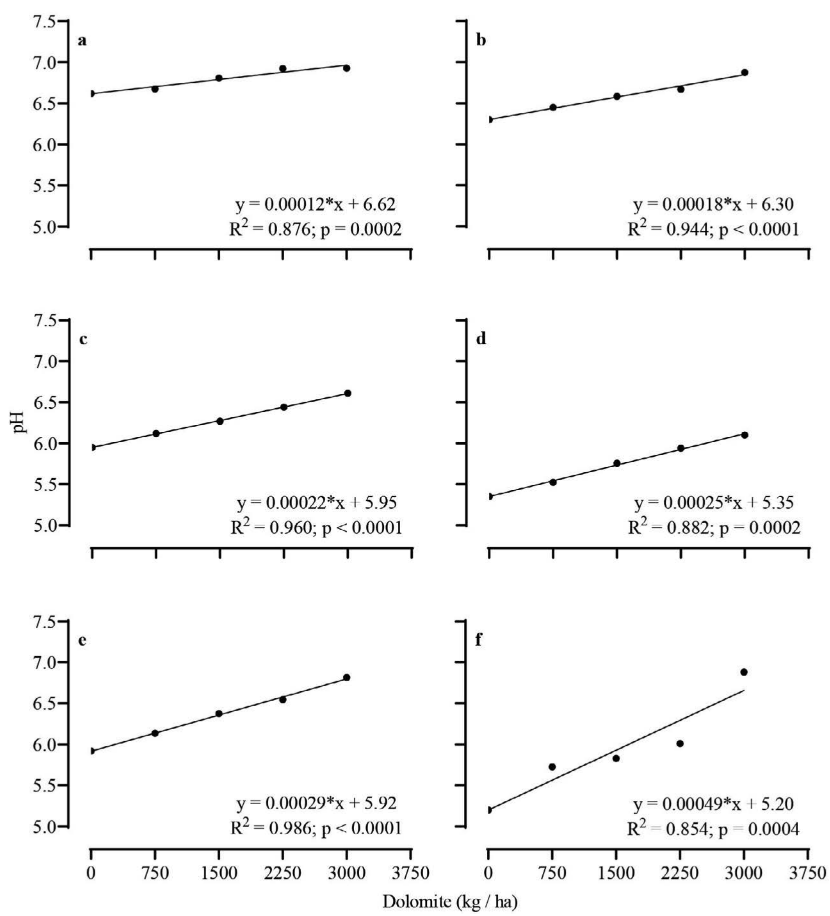

Figure 1. Values of pH in soils of low (a, b), medium (c, d, e) and high (f) response in the presence of the dose of double calcium-magnesium carbonate (Dolomite), in soils belonging to the Inceptisol order from the locality of Quevedo, Los Ríos – Ecuador. The full circles (•) represent the average of two data (n=2).

The resistance of the soils to change pH has been associated dominantly with the content and type of secondary minerals (silicate, non-silicate, crystalline and amorphous) and to the organic matter in different degrees of stabilization, which make up the active solid fraction of the soils and which differs between the different types of soils (Sanchez, 2019). The variability inherent to the active solid fraction of the soil confers to each soil a specific resistance in face of the increase or reduction of the pH; thus, in soils where the same increase of pH is sought, different doses of the same liming material are required, as evidenced in this study where Soil A had a $BC_{Dolomite}$ of 1/0.00012, while in the Soil F the $BC_{Dolomite}$ was 1/0.00049 (Figure 1).

CONCLUSIONS

The buffer capacity of the soil in the presence of the addition of dolomite ($BC_{Dolomite}$), constitutes a viable method to estimate the liming requirement of the soils, since the $BC_{Dolomite}$ changes in the different soils within the limits evaluated in this study. This evidences that it is a method which is acceptably sensitive to the complexity of the clay fraction and the organic matter of the soil. Stemming from the fact that $BC_{Dolomite}$ is the inverse of the resulting slope when evaluating the pH_{H_2O} in function of the dose of dolomite, it is suggested that the requirement of dolomite ($R_{Dolomite}$) is equal to the product of the desired increase in pH (ΔpH) and the $BC_{Dolomite}$ $\left(R_{Dolomite} = \Delta pH \times CT_{Dolomite}\right)$.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Amaranth production in Tulyehualco Xochimilco, Mexico City

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ABSTRACT

Objective: To know the form of production and commercialization of amaranth in Tulyehualco, Xochimilco, to identify and propose improvement actions.

Methodology: During 2010-2019, amaranth was cultivated in at least 11 states in Mexico, while in Mexico City it is grown in the municipalities of Xochimilco, Milpa Alta and Tláhuac. Xochimilco stands out due to harvested area and production, with 82.9 ha and 91.7 t which represents 60.4% and 55.6% respectively. The information was obtained through the application of a survey through non-probability sampling for convenience, with the selection criterion of individuals who were willing to be surveyed, and the survey was applied from September to December 2019, to n=35 producers, n=3 marketers and n=4 transformers. Amaranth production is carried out in two ways, by means of chapin and directly, and due to its traditional way of producing the crop is ancestral in those areas, so there is a millenary knowledge of the families that are dedicated to planting the crop.

Results: Amaranth production has a positive cost benefit ratio R (B/C) although production is better in direct sowing. Planting with a seedbed (Chapin) has higher costs and yield, however, this does not compensate the producer in profits.

Conclusions: For a potential impact at the level of amaranth production, the adoption of technologies related to density, nutrition and technical recommendations for pest and disease control is necessary.

Keywords: Chapin, production costs, profitability.

Image by Petra Göschel at Pixabay

INTRODUCTION

Amaranth (*Amaranthus* spp.) is one of the most ancient crops in Mesoamerica, as the first data of this plant date back to ten thousand years ago and its role in the diet was as important as corn and bean (Corona González *et al.*, 2019). In Mexico, it faces various problems, especially in its production phase regarding a low technological level, native varieties of low yield, and unfavorable agronomic characteristics, such as late maturation, very tall plants, variation in color of plant and seed (Ruiz Hernández *et al.*, 2013), etc. During 2010-2019 amaranth was cultivated in at least 11 states in Mexico, among which Puebla, Tlaxcala, Estado de México, and Mexico City stand out with an average of 2,108 ha, 1,153, 285 and 137 ha of surface harvested annually (Sistema de Información Agroalimentaria y Pesquera, 2020). The contribution of production has been of 50.6% from Puebla with 2,794 t on average, 30.3% Tlaxcala with 1,672 t, 11.10% Estado de México with 613 t, and 2.9% Mexico City with 161 t (SIAP, 2020). Amaranth is cultivated in Mexico City in the mayoralties of Xochimilco, Milpa Alta and Tláhuac, with Xochimilco standing out thanks to its harvested surface and production with 82.9 ha and 91.7 t which represents 60.4 % and 55.6 %, respectively; during 2010-2019 the average annual growth rate (AAGR) of the surface harvested was 0.27% while the AAGR of production was 1.34%; however, the highest yields are from Tláhuac with 1.30 t ha^{-1} (SIAP, 2020), although the AAGR of the surface harvested is -10.8 %.

In Tulyehualco amaranth is transformed artisanal and industrial way for its commercialization (Ramírez Meza *et al.*, 2017). It is consumed as popped grain from which products such as alegrías, cereal, granola, atole, pinole, marzipan, etc., are elaborated. There are also snacks, bread products, cookies, and amaranth flour, which is used in the elaboration of soups, cakes, and breads (Escobedo *et al.*, 2012). De Jesus Contreras *et al.* (2017) mentioned that amaranth production in Tulyehualco is a representative activity of local culture, and they highlight its transcendence as building element of territorial identity and historical depth, with peculiar and traditional agricultural practices characterized by a sustainable management of natural resources. Velarde (2012) points out that in Tulyehualco, amaranth is a historical food product that resulted from the dynamics of a particular territory. Derived from its importance in this region, the objective of study is to understand the form of production and commercialization of amaranth in Tulyehualco, Xochimilco, to identify and suggest improvement actions.

MATERIALS AND METHODS

Xochimilco is located on geographical coordinates 19° 19’ north and 19° 09’ south of latitude North; it represents 7.9% of the surface of Mexico City. It has eight main localities: Tepepan, San Luis Tlaxialmateco, Tulyehualco, Xochimilco, San Gregorio Atlapulco, Santa Cruz Acalpixca, Santa María Nativitas and San Francisco Tlalnepantla. In 60.8% of the territory, there predominates a subhumid temperate climate with summer rains, with medium moisture, followed by subhumid temperate with summer rains, with higher moisture in 26.5% of the territory (Instituto Nacional de Estadística Geografía e Informática, 2014). The study was conducted in the locality of Tulyehualco, where there are a total of 100 producers cultivating amaranth. The information was obtained through the application of a directed survey. The use of this technique is justified when there is not enough informative material about the aspects that there is an interest in researching, or when the information cannot be obtained through other techniques (Rojas Soriano, 2013). A non-probabilistic sample from convenience was carried out, with the selection criterion of individuals who were willing to be surveyed, and it was applied from September to December of 2019, to 35 producers, 3 marketers and 4 transformers. Calculating the profitability was done by obtaining the average production costs on which they incur during the production process. The costs were divided in two parts: direct costs and indirect costs (Swenson & Haugen, 2012). Within the direct costs, the costs of inputs and production means were included, such as seed, fertilizers, workforce, and opportunity cost of the investment. Land rental and general expenditures were included in the indirect costs. To determine the profitability, the following algebraic expressions were used, based on economic theory (Krugman & Wells, 2006; Samuelson & Nordhaus, 2009).

$$TC = PxX$$

Where TC=Total cost, Px=Price of the input or activity, X and X=Activity or input.

The total income per hectare is obtained by multiplying the crop's yield by its market price. The algebraic expression is:

$$TI = PyY$$

Where TI=Total income (\$ ha^{-1}), Py=Price of crop market Y (\$ t^{-1}); Y=Yield of the crop (t ha^{-1}).

Finally, the profitability is calculated with the following formula:

$$Profitability = TI - TC$$

RESULTS AND DISCUSSION

The producers were in an age range of 35 to 79 years, with an average of 57 years, although it was found that the age range among amaranth producers was 20 to 79 years, with average age of 48 in the study conducted in the states of Puebla, Tlaxcala, Morelos, and Hidalgo (Ayala Garay *et al.*, 2016), which shows that the older producers are those from Mexico City. Of the total people interviewed, 5% were women and the rest men; regarding education, it was found that 22% of the producers have concluded higher level studies, another 22% middle level, and 56% basic level; and small-scale land ownership of 1.5 ha predominates.

Amaranth production process in Tulyehualco, Xochimilco

Land preparation is mechanized. During sowing, the seed that is used is the one collected from previous cycles, which producers store in sacks in their homes. Ramírez Meza *et al.*

(2010) found that the producers safeguarded the Teuhtli Ecological Conservation Zone through transmission of their traditions, identity and knowledge of their environment from generation to generation, and, at the same time, the cultivation of amaranth and its genetic diversity. Moreno Velázquez *et al.* (2005) mention that it is a common practice among producers to select in the field the panicles with adequate phenological characteristics for their common use as seed, and based on this practice, more than 50% of the surface cultivated in Mexico with amaranth is sown with seed from the previous harvest, without taking into consideration its physical, physiological, and sanitary quality. On average, six kilograms of seed are used to sow a hectare. Only 5.6% of the producers use selected seed. The topological arrangement is furrows with width of 0.40 m, at 0.60 m and 0.30 m of between each sowing hole, in which a variable number of seeds is deposited with the purpose of ensuring that several plants germinate and grow in each hole. This is so that clearing is done 30 days after sowing, which consists in uprooting the smallest plants and leaving three, four or five plants, depending on the productive logic of each producer. Of the interview respondents, 76.7% mix the seed with soil or dry livestock manure before sowing, with the aim of seeds not being eaten by birds or removed from the furrow as a result of rain or wind; this is because the seeds are placed in the sowing hole without being covered, since from their experience if the seed is covered the number of plants that emerge is very low, with which they run the risk of needing to replant. Regarding fertilization, the producers carry out two applications of fertilizer; the first is at the time of sowing and the other 45 days after emergence.

Concerning technical assistance, 56% mention that the lack of advice against pests is the main problem that they face, since they ignore how to combat them. Pérez Torres *et al.* (2011) reported that *Hypolixus truncatulus* (Fabricius) (Curculionidae) was identified in Tulyehualco, and they mentioned that the larvae causes damages in the stem, by making a series of galleries along it and therefore the necessary nutrients are not absorbed, since they do not reach the apical tissues which are weakened until they break; and this happens between the months of September and November, when the plant is undergoing the beginning of anthesis (pollen release) and its maturation begins.

Forms of amaranth production in Tulyehualco, Xochimilco

Ramírez Meza *et al.* (2017) mention that presently two types of amaranth sowing are known:

1. Indirect sowing, which is characterized by generating first the seedbeds (called chapines) where the seed is sown and germinated, to later perform the transplant (72% of the producers). Espitia Rangel *et al.* (2010) mention that in Tulyehualco, Mexico City, the crop is rainfed, it is grown on the skirts of nearby hills and goes through two stages: seedbed and transplant. The seedbed is prepared from the end of April to the beginning of May, to carry out the transplant at the beginning of the rainy season, which habitually takes place since June. The seedbed preparation is done through the chinampa system and for that purpose the beds are established in plots near the canal, with size of 5 to 10 m width and 100 m length. The next day, when the bed is slightly

dry, it is cut into small squares (chapines) and it is sown. In sowing with chapin, inputs are used such as bokashi (organic fertilizer that is obtained from the fermentation of dry materials that are conveniently mixed; the nutrients obtained from the fermentation of the materials contain higher and lower elements, which form a complete fertilizer superior to the chemical fertilizer formulas) and sulfo-calcic soup, where the active ingredient is calcium sulfide (The Food and Agriculture Organization, 2011), which serves to control pests while in direct sowing it is only the manual application of cattle manure as fertilizer.

2. Direct seed drilling with high population density (28% of the producers), where the seed is dropped without leaving any space, covered lightly and the plants grow together, the distance between furrows is 60 cm and 4 to 6 kg of seed per hectare are required (Ramírez Meza *et al.*, 2017).

When it comes to fertilization, 6.6% of the producers do not apply any type of fertilizer; 15.6% apply only chemical fertilizers, generally 50 kg ha^{-1} of diammonium phosphate (18-46-0) 20 days after sowing and 50 kg ha^{-1} of urea (46-0-0) 60 days after sowing; 77.8% of the survey respondents combine chemical and organic fertilization which consists in average applications of one ton of livestock excretes per hectare before sowing, 50 kg ha^{-1} of diammonium phosphate and 50 kg ha^{-1} of urea following the same calendar of farmers that only conduct chemical fertilization. The organic fertilizer most frequently used is livestock defecation, primarily from cattle.

The families that do not own livestock (43.7%) purchase the fertilizer in other neighboring municipalities. Weed control is conducted manually and periodically during the entire cultivation cycle. From the middle of October to the end of November, once 90% of the plants reach maturity, the panicle is cut, and it is left for eight days in the open. The cut is done with a sickle or machete and left to dry so that now of seed threshing the seed is detached more easily. During the time when the panicles are in contact with the ground there is a risk that a certain amount of grain is lost whether from detaching or from rotting caused by fungi of the *Thecaphora* genus. Threshing is carried out generally in December, although some producers do not thresh until the beginning of January. Of the interview respondents, 22.6% thresh with the traditional method, which is done manually or with the use of load animals. This method consists in stepping on or whipping the dry panicles with rods, on cloths or canvases, for the seed to detach; then the seed is sifted to remove the dust and hay known as chaff. With the traditional method, threshing a hectare is done approximately in one week, although it depends on the number of workdays and the climate conditions, since in cloudy days and with high relative moisture the seed detaches with more difficulty. In contrast, 77.4% of producers use harvesting machines, destined for other crops such as sorghum (*Sorghum* spp.) from other municipalities or even from other states, to save time and work, since with this machinery a hectare sown with amaranth is threshed in one hour and less workdays are used. On average, five workers per hectare are employed to introduce the dry panicle manually into the harvester and to pack the grain. Since the harvesting machines are not designed for the amaranth grain, a certain amount of grain from the production is lost when it falls to the ground while the harvester advances. The average yield in the zone under usual agroclimatic conditions is 1.2 t ha^{-1}, under direct production and 1.35 t ha^{-1} of production through chapin, while the national average was 1.66 t ha^{-1} during 2016-2019 (SIAP, 2020).

Amaranth production costs in Tulyehualco, Xochimilco

For both types of sowing, the amaranth production costs were obtained (Table 1).

For the producers who cultivate with the help of chapines (seedbeds), they represent 13.7% of the total expenditure; the usefulness of employing chapin is to achieve the germination of amaranth under better conditions, without competition from weeds for nutrients, and when it reaches a height of 10 cm it is transplanted into the plot. In both cultivation methods there is an expenditure in workdays that represents on average 34.7% of the total costs, which reflects the great use of labor to carry out the activities that include chapin, weeding, sowing, fertilization, and harvest. Similarly, Ayala Garay *et al.* (2014) point out that in the sates of Puebla, Tlaxcala and Morelos, the cost structure per hectare of amaranth has expenses in manual labor as the most important segment, which represented 37% of the total cost on average, followed using inputs (21%) and mechanized tasks (19%). The cultivation of amaranth demands labors greatly, which makes its production process more expensive, and this is reflected in the cost structure.

Commercialization of amaranth

Regarding commercialization, 40% is carried out with stockpilers, and 60% of the products in the zone transform amaranth into various byproducts such as brittle, cookies, wafers and breads, which are traded directly in shops set up in their households or in semi-permanent stands on the streets. The producers who trade their harvest with local transformers sell at a low price, with the argument that it is a native seed. However, the

Table 1. Production costs of direct sowing and sowing with chapin, cycle 2019 (USD).

Concept	Planting con chapin	Direct planting
Land preparation ($/ha)	90.51	115.65
Chapin preparation ($/ha)	150.85	0.00
Inputs ($/ha)	176.50	278.58
Wages ($/ha)	394.73	289.14
Harvest($/ha)	291.65	173.48
Total Cost ($/ha)	1104.24	856.85
Yield t/ha	1.35	1.20
Price ($/t)	930.26	930.26
Income ($/ha)	1255.85	1116.31
Utility ($/ha)	151.61	259.47
Cost ($/t)	817.96	714.04
Profit per t	112.30	216.22
Benefit/Cost	0.06	0.07

Source: Prepared by the authors with information gathered in the field during October-November 2019. Prices from 2019.

transformers stockpile the production from Puebla and Morelos where they buy it at a higher price than in Tulyehualco, although the seed pops on average in 80%. However, there is a transformer who gives extra payment of 15% over the price to local producers since he does it with the intention of giving value to the native seed of the zone and supporting the producer to recover his investment.

Problems with amaranth in Tulyehualco

Regarding its cultivation, it is done under rainfed conditions, which is why it is necessary to understand water capture techniques and, with these, to have water available for auxiliary irrigation; in addition, the producer faces low yields per hectare, since lacking a production technology appropriate to the agroclimatic zone, sometimes producers ignore what to use to combat pests and diseases. Ayala Garay *et al.* (2014) mention that technical problems which prevail in amaranth cultivation make it necessary to elaborate a program for technology transfer and continuous training that detects the needs of the producer with the aim of improving production and yields, as well as minimizing risks to the crop. Something else that threatens the cultivation zone is the growth of the urban sprawl, invading cultivation lands since it is attractive for the producer to sell his plot for housing due to the income it generates, resulting partly from ageing of the people who are devoted to the farmland, who are over 55 years and because few young people are involved in farming, with amaranth plots being left without generational replacement. Ayala Garay *et al.* (2020) mention as part of their conclusions that an institutional transversal strategy is required to promote the cultivation and consumption of amaranth in Mexico, highlighting that, just as it is tolerant to droughts for cultivation and production, it is also a functional food that in addition to nourishing can prevent certain diseases due to its high content of essential amino acids and its content of bioactive peptides.

CONCLUSIONS

Amaranth production in the locality of Tulyehualco ought to be recognized because of its traditional form of production, where it stands out that its process follows ancestral techniques such as chapin. In the present scenario, primary production has a high demand for labor, particularly in sowing and harvesting, which generates employment in the region and rootedness of families in the region; however, mechanization is required. It is necessary to design new institutional channels to perform the transference of the existing production technology to the amaranth cultivation areas, with the objective of improving production, controlling phytosanitary problems, increasing yields, and diversifying its use and consumption.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Incorporation and management of improved avocado in the orchards of Tétela del Volcán, Morelos, Mexico: Case study of Gupo FRUFIDET

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ABSTRACT

Objective: To analyze the process of incorporating improved varieties of avocado (*Persea americana* Mill.) and its effect on the diversity, management and use in 54 orchards belonging to 25 members of the Rural Production Society of C.V. Grupo FRUFIDET in Tétela del Volcán, Morelos, Mexico.

Methodology: A survey was applied to all members of society considering the characteristics of the orchards and the process of incorporating improved varieties of avocado to their orchards. The diversity associated with avocado cultivation was obtained through participant observation and a survey. A typology of orchards was carried out with a principal components analysis.

Results: The improved avocado has replaced the peach crop by 67%, plum 12%, corn and bean 12%, pomegranate 6%, and fig 3%. Three types of orchards were found: those that have replaced the previous crop with improved varieties of avocado (17%), those that have introduced avocado as one more species to their orchard in combination with other fruit trees (53%), and those which are maintained with fruit trees and Mexican varieties of avocado (30%).

Conclusions: The improved avocado varieties have been incorporated by 100% of the members of the organization. Their incorporation has caused the displacement of Mexican varieties of avocado and other fruit trees.

Keywords: Mexican varieties of avocado, improved avocado, diverse orchards.

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INTRODUCTION

Avocado (*Persea americana* Mill.) is the second highest agrifood product sold by Mexico, with a surface sown of 241 140 ha in 2020, and a production of 2 388 615 t, maintaining constant growth (Banco de México, 2018; SIAP, 2021). The Association of Avocado Producers and Packers in Mexico (*Asociación de Productores y Empacadores de Aguacate de México*, APEAM) has exceeded one million tons sent to the United States, positioning Mexico as the main producing and exporting country of avocado (APEAM, 2021).

Image by Daniel Dan outsideclick at Pixabay

The state of Morelos is the fifth avocado-producing state with a surface of 5731 ha and a production of 50 402 t, in 13 municipalities, among which Ocuituco and Tételа del Volcán stand out with a surface of 2082 and 1820 ha and a productivity of 17 814 and 16 867 t, respectively. Together, they contribute 89% at the state level (SIAP, 2020).

According to SIAP data, 715 ha were found in 2003 sown with the avocado crop in the municipality of Tétela del Volcán increasing its surface to 1820 ha in 2020.

Avocado has become a crop of economic and social importance in the producing zones, although its expansion in some cases has environmental effects such as degradation and contamination of soils, inefficient use of inputs, and water contamination (Gavito Pardo *et al.*, 2011). The generation of direct and indirect jobs is attributed, positively, to the increase in surface sown with avocado; however, 690 ha of forest zones are deforested annually for its establishment. This generates important implications in the functionality of ecosystems (INIFAP, 2012).

The Mexican varieties of avocado (native avocado) play an important role for the success or failure in the establishment of commercial orchards of this crop (Castro *et al.*, 2003), since they are used as rootstock to obtain a quality plant (Castro & Fassio, 2015). This places at risk the genetic diversity of avocado and causes the disappearance of native and semi-wild materials (Sánchez, 1999).

The increase of the avocado crop, in monocrop, causes the loss of diversity in producing zones (Rosset & Martínez, 2016). This restricts the appearance of weeds and their role in erosion control and nutrient recycling in the soil, nitrogen fixation, by some legumes. Likewise, their use as food or medicine and the attraction of pollinators is limited (Merlín-Uribe *et al.*, 2014).

This study analyzed the process of incorporation of improved varieties of avocado (Hass, Méndez, Jiménez and Fuerte) and its effect on diversity, management and exploitation of the orchards, through their characterization and categorization, taking as case study the orchards that belong to the producers of Group FRUFIDET (Fine Fruits and Byproducts from Tétela del Volcán, *Frutas Finas y Derivados de Tétela del Volcán*).

MATERIALS AND METHODS

Tétela del Volcán meets the current of the Amatzinac Ravine, which originates from the skirts of the Popocatépetl Volcano, northeast of the state of Morelos. Its geographical location is 18° 57’ latitude North and 98° 14’ longitude West, at an altitude of 2040 masl. There are many climates: temperate sub-humid with summer rains, of highest humidity (73.4%), semi-cold sub-humid with summer rains, of highest humidity (19.37%), semi-warm sub-humid with summer rains, of medium humidity (6.48%), and high altitude cold with marked winter (0.75%) (INAFED, 2020; INEGI, 2009). Tétela del Volcán has an approximate surface of 98.61 km^2 distributed in 3035 ha for agricultural use and 6602 ha for forest use. The soils are deep, rich in minerals and nutrients. The fertility and organic matter content are limited, most of the orchards present slopes over 20% given the uneven orography of the region, in addition to soil erosion (Márquez Berber & Colinas León, 2019).

The study was conducted with the Rural Production Society of C. V. Grupo FRUFIDET, made up of 25 producers/ras. The field work was carried out in March and April 2021, through a mixed methodology strategy that involved all of the members. A survey was applied through a questionnaire with the following sections: general data, characteristics of the orchards, adaptation of improved varieties of avocado, management of orchards, and diversity associated to the avocado crop. The data were processed through the software Excel 2010 and SSPS version 24 for the elaboration of graphs, and a principal components analysis. In addition, participant observation, visits and tree registry were conducted within each of the orchards.

RESULTS AND DISCUSSION

The society started in 2003 with 320 producers, and currently there are 25 from which 14 have been in it since the beginning, while the rest became integrated through time. Grupo FRUFIDET is made up by 22 men and three women with an average age of 54 years, a minimum of 20 and a maximum of 89. The grade of studies of the producers is four with Bachelor's degree, six with high school, five with secondary school, five with primary, and five do not know how to read and write.

Agriculture is the occupation that 76% of the members carry out as main activity, and 24% as second option. The agricultural surface as a whole has an extension of approximately 60 ha, with an average of 2.4 ha, and two orchards per producer on average. The minimum surface of a producer is 0.25 and the maximum four ha. However, because of land fractioning, the ownership of the orchards varies, from those who have two (11), one (6), three (6) and four orchards. The producers with two or more orchards cultivate improved varieties of avocado as monocrop and in association with other crops.

Avocado in the orchards of Tétela del Volcán

In the survey the producers indicated that they have historically cultivated different fruit trees (peach, plum, blackberry, sweet granadilla, pear, fig and avocado), although the fruit trees of greatest profitability and persistence in time were peach orchards and then the improved varieties of avocado. Peach was a crop of high economic importance at the local and regional level, allowing for members of the Grupo FRUFIDET to recognize themselves as producers and traders of this fruit. In this regard, Chávez & Chávez (2006) mention that in 2004 they had a record of 2500 ha with peach crop in the municipalities of Tétela del Volcán, Ocuituco, Zacualpan de Milpas, and Tlacotepec, of which 850 ha correspond to Tétela del Volcán (SIAP, 2004).

In 2005 the produces from Tétela del Volcán began to have problems with pests and diseases, among which the following stand out: a) damage from nematodes (*Dorylaymus*, *Tylenchus*, *Aphelenchus* and *Pratylenchus*) (González Cortés *et al.*, 2010; Meza Durán, 2019); b) San Jose scale (*Quadraspidiotus perniciosus*), and c) white root rot (*Armillaria mellea*). Another frequent problem is the presence of aged plantations and the lack of an integral management of the crop. The previous problem caused for producers to seek other alternatives for growth (*Eng. Rolando Mendoza, Personal communication, April 19, 2021*). They incorporated improved varieties of avocado gradually in their orchards. Eight producers did it between

1980 and 2000, five from 2001 to 2005, and the last producer of the group who introduced avocado did it in 2017, with which 100% of the producers cultivate improved varieties of avocado (Figure 1).

The surface with which the producers began growing avocado was: 0.5 ha (12); 0.6 to 1 ha (eight); 1.1 to 2 ha (four); and 2.1 to 5 (one); 17 producers mentioned that they began testing with a few trees. According to the information obtained, from the years 2014 to 2019, 68% (17) of the producers mentioned having increased the sowing surface with improved varieties of avocado, 20% (five) conserve the same surface area that they started with, and 12% (three) decreased it due to issues of adapting to the place or presence of *Phytophthora cinnamomi*. Currently, the improved varieties are represented as follows: 46% Hass, 36% Jiménez, 15% Méndez, and 3% Fuerte.

Tree composition of orchards

The producers have a total of 54 orchards, from which 85% include improved avocado, followed by 9% plum, 4% fig and another 2% is represented by avocado of the Mexican variety. The improved varieties of avocado are the main source of agricultural income for members of Grupo FRUFIDET due to the permanent commercial demand for the product and because the producer schedules the cuts and the harvest according to their economic needs, which agrees with Rubí *et al.* (2013) who mentioned that the adoption of Hass avocado from producers of the south zone of the state of Mexico, happened because of its easy commercialization and high acceptance in the market, resulting in important economic revenues for producers.

From the orchards, 14% (seven) do not have improved avocado varieties; seven maintain a monocrop with plum (five) and with fig (two). In contrast, there is a single orchard (2%) that conserves Mexican avocado varieties. Generally those who have a single orchard present tree diversity, with the exception of one producer who has improved avocado in monocrop. However, those who have more than one orchard manage improved avocado varieties, Mexican varieties, and other fruit trees in different modalities of the crop (monocrop, associates, interspersed, in lines, etc.).

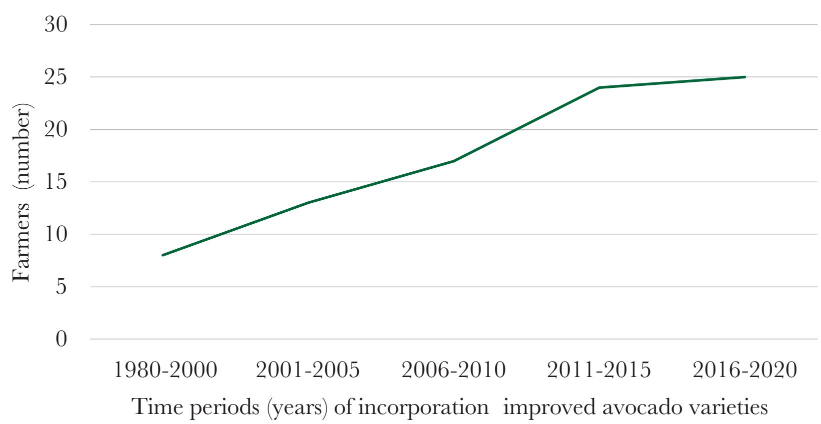

Figure 1. Process of incorporation of improved avocado in the orchards of 25 producers from Grupo FRUFIDET during the period of 1980 to 2020. Source: prepared by the authors with field data.

The 46 orchards present six types of arrangements (Figure 2): a) Orchards with improved varieties of avocado in combination with other fruit trees (peach, plum, apple, walnut, Colombian pomegranate, pear, blackberry, custard apple, lime, black cherry, loquat, star fruit, guava, orange, papaya and quince) and annual crops (oat, maize and pea); b) with improved avocado varieties in combination with Mexican varieties, other fruit trees, and annual crops; c) with improved avocado; d) six orchards with Mexican varieties in combination with improved varieties; e) with fruit trees in monocrop (five with plum, two with fig, and one with Mexican variety of avocado).

According to Guzmán *et al.* (2017as the center of origin of avocado (*Persea americana* Mill.), the geographic dispersion of improved varieties of avocado is attributable to the presence of Mexican varieties which are adapted to each local environment, and this has reduced the populations of Mexican varieties to give rise to improved ones, since the first are used as rootstock because they have genes resistant to pests and diseases. The substitution of Mexican varieties for improved ones, plus the destruction of habitats, place at risk the Mexican varieties of avocado and diversity in general (Rincón-Hernández *et al.*, 2011; Sánchez, 1999).

The importance of the diversity of Mexican varieties of avocado consists in their use to form family orchards, to later select seeds that are used in commercial nurseries as rootstock (Bellón *et al.*, 2009). However, scarce interest has been shown in the classification and conservation of germplasm that allows the protection of this national heritage of diversity, and the conservation of different genotypes of Mexican varieties that nowadays are threatened when they are substituted by improved varieties (Gutiérrez-Díez *et al.*, 2009), which places them at risk of disappearance. In this regard, at the national level, INIFAP and the Salvador Sánchez Colín-CICTAMEX Foundation are the ones that preserve part of the genetic reservoir (Bellón *et al.*, 2009).

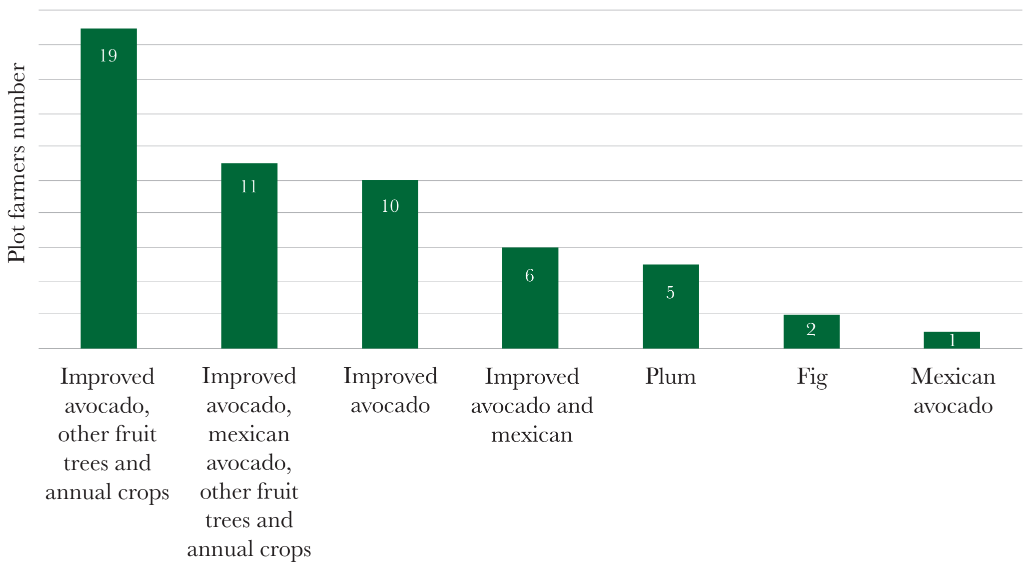

Figure 2. Diversity of orchards in Grupo FRUFIDET, 2021. Source: prepared by the authors with data from field work.

Management of the orchards

To conduct management of the orchards, 15 of the producers (60%) resort to hired technical counseling, particularly for the avocado crop. Eight of them are advised by a technician from the agrichemicals store, five receive it from an independent technician and two from a technician from the State Center of Plant Health from the state of Morelos CESVMOR. The main practices for which they receive support, in order of importance for the producer are: nutrition, pest and disease management, pruning, establishment of the orchard, plant reproduction and commercialization. The specialized technical assistance is useful to obtain quality production and to reduce phytosanitary problems that sometimes affect the sale of avocado (Rubí *et al.*, 2013).

The management practices of the orchards are generally conducted with family labor, although in practices such as application of nutrients, fertilizers, pruning and harvest, 80% of the producers use family and hired labor, 16% only family labor, and 4% depend on hired labor.

Many of the jobs generated by avocado are temporal jobs, particularly at the time of the harvest, although in recent years the possibility has come up of foreign buyers taking their cutters and not hiring local people, generating economic instability for the families, which agrees with what was reported by Macías-Macías (2009).

When it comes to management of the water resource, 23 producers have water for irrigation, 22 do it with water from volcano ice thawing, and one owns a well. For their part, two producers do not have irrigation and take advantage of rainstorms.

Diversity associated to avocado

Merlín-Uribe *et al.* (2014) mentioned that there is evidence that weed management increases the productivity of the crop, in addition to being auxiliary in minimizing the presence and amount of pests. However, Escobedo-Cruz *et al.* (2017) mention that weeds affect the crops, due to competition over water, nutrients and light. However, conserving it as organic padding favors the soil.

In the diverse orchards, it was found that producers have other usable species, so that weeds are allowed in 72% of the orchards, 8% have another annual crop in the space between lines of avocado, and 20% mentioned using herbicides to keep the soil clean.

The usable species that were part of the inventory of the orchards are used as: a) Food: violettas (*Anoda cristata*), amaranth (*Amaranthus* sp.), pomegranate (*Punica* sp.), turnip (*Brassica rapa*), thorny amaranth (*Amaranthus spinosus*), jocote (*Spondias purpurea*), purslane (*Portulaca oleracea*), huauzontle (*Chenopodium nuttalliae*) and fig-leaf gourd (*Cucurbita ficifolia*); b) Medicinal: rue (*Ruta graveolens*), basil (*Ocimum basilicum*), lavender (lavándula) and kidneywood (*Eysenhardtia polystachya*); c) Boundary or firewood: cedar (*Cedrus* sp.), oak (*Quercus* sp.), ocote (*Pinus moctezumae*) and oyamel fir (*Abies religiosa*); and d) Fodder: wild radish (*Raphanus raphanistrum*).

In general those who leave the weeds do it to favor bees and other pollinators, according to their testimonies. The conservation of other species in the orchard, which are also used, signals the possibility of conducting agro-forestry practices that can be an option to increase the productivity and profitability of the orchards. It has been proven that by having more biological diversity, more environmental and economic benefits are obtained (Montiel-Aguirre *et al.*, 2008).

Characterization

The characterization of producers carried out considered the following variables: surface and slope of the orchard, presence of improved varieties of avocado, Mexican varieties, other fruit trees, irrigation, and usable species. The analysis differentiates two groups of orchards, where diversity is one of the most important variables (Figure 3).

Group one or diverse orchards (76%) manages more than one species of fruit trees, having improved avocado varieties (20-450 trees), and in addition manages other fruit trees and Mexican varieties. They have surfaces between 0.2 and 1.5 ha, gentle to pronounced slopes (more than 20%).

In the various orchards the risks from climate effects can be decreased, the soil fertility favored, the erosion decreased, and the agricultural income diversified; this agrees with what was shown by Nataren-Velazquez *et al.* (2020).

The members of group two, which is equivalent to non-diverse orchards (24%) have surfaces of 1 to 2.8 ha, gentle to pronounced slopes over 20%, trees of improved varieties (from 500 to 625 trees), irrigation systems and weed control.

CONCLUSIONS

Improved avocado varieties have been incorporated by 100% of the members of Grupo FRUFIDET, and their integration to the orchards has been as monocrop, associated crop or interspersed with other tree species, annual crops, and other useful species. The incorporation of improved avocado varieties has caused the displacement of Mexican varieties of avocado, due to their importance as rootstock; however, they are placed at risk

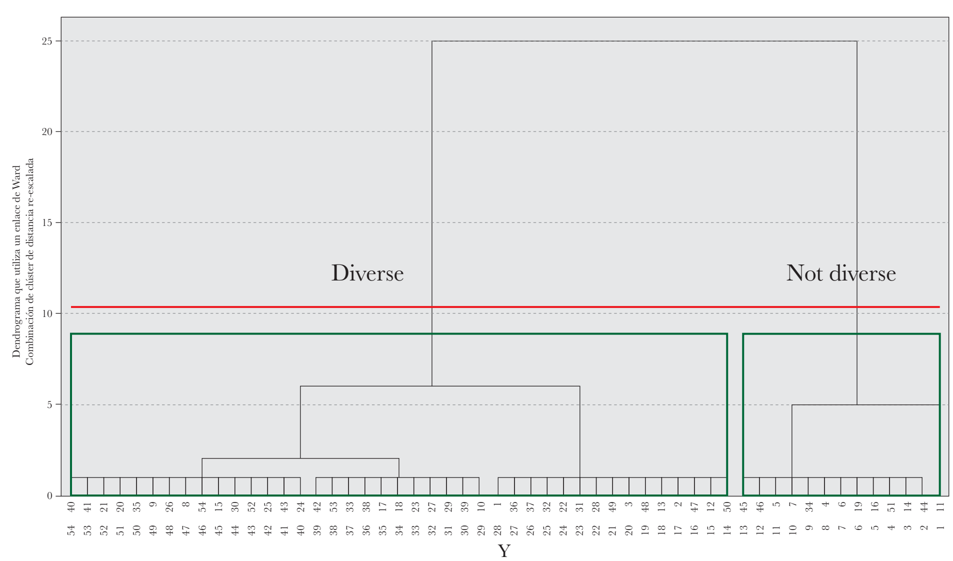

Figure 3. Typology of orchards from Grupo FRUFIDET.
Source: Prepared by the authors with data from field work.

from not being valued as genetic reservoir. The members of Grupo FRUFIDET manage diversified systems as a strategy for the exploitation of other species that are used as food, medicinal, boundaries, firewood; in addition, they avoid erosion and favor pollinators.

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Technological parameters of agroindustrial concern of prickly-pear cactus (*Opuntia* spp.) in “Cristalina” and “Pelon Blanco” varieties

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ABSTRACT

Objective. To test and verify the main physical attributes of fresh cladodes of the prickly-pear cactus (nopal) Cristalina and Pelon Blanco varieties; and the flours obtained after they are dried and ground.

Design/Methodology/Approach. Physical characteristics were determined using proven and reliable tests. Data shall serve to explore the potential of this material to be used in basic and complex processes; considering the varieties requierements for growth space and quality parameters for storage. Along with the interaction with processing devices and equipment for proper handling while manufacturing new products.

Results. Physical characteristics of the two nopal varieties showed slight differences in the measured parameters, except length, width and electrical conductivity.

Limitations/Implications of the study. Researchers did not participate in the production of the evaluated *Opuntia* spp. varieties. Physical characteristics of the varieties were recorded at the time when the cladodes were separated from the plants.

Findings/Conclusions. With the procedure proposed, the mechanical transport of the cladodes into the facilities is improved. In addition, it generates less wear of the equipment, and reduces the costs of maintenance and handling. The quality of the flour from each variety was adequate to allow a safe storage.

Keywords: food agroindusty, agricultural products, physico-chemistry, measurement techniques, prickly-pear cactus (nopal).

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INTRODUCTION

The prickly-pear cactus or nopal (*Opuntia* spp.) is a plant species with great roots in arid areas where it is difficult to produce conventional crops, due to adverse agroclimatic conditions, such as recurrent droughts and shallow soils and low fertility (Méndez *et al.*, 2004). In this era of modernity and innovation, a task of agroindustry is to conduct viable processes to transform agricultural and livestock products, to estimate and promote the

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manufacturing sector, as an alternative for food security and derivative agroindustrial products; as well as for generating strategies to facilitate their transfer from the field to the dinner table (Austin, 1987).

An additional resource, based on energy efficiency principles, is achieved through the grinding of the vegetable components until reaching the point of flour. With this process, an excellent stabilization of the minerals is achieved. This becomes relevant because nopal (*Opuntia* spp.) is a plant in which only cladodes of one year (or pencas) are used, but the other cladodes (2 years-old and onwards) are not considered useful in traditional agriculture (Reyes-Agüero *et al.*, 2005).

This proposal is due to those inefficiencies in the current management scenario, which is subject at least to factors such as natural variation in the production either quantity and quality of biomass in nature; increase in such nature variability conditions due to the increase of adverse climatic effects; increase in the need to produce food and biological raw materials for other productive sectors due to the increase in world population; available technologies for processing that adds value to the agricultural organic products; and the limitation to access to greater amount of natural resources. Therefore, it is essential to pay greater attention to use the entire plant.

Some possible beneficiaries of this sector would be; food and beverages agroindustry for human and animal consumption; the cosmetic industry, the pharmaceutical industry, the synthetic and semi-synthetic food supplements, the production of natural additives, as well as: the construction, energy, producers of inputs for agriculture, tourism, the textile industry, art, among others. The fruits and the pencas contain valuable components, among which they stand out; fiber, hydricoloids (mucilage), pigments, vitamins and minerals, highlighting vitamin C. Additionally, antioxidant properties are attributed to them. On the other hand, several studies have found that cladodes are an important source of fiber, calcium and mucilages. These three components are necessary to integrate a healthy diet (Sáenz, 2004).

To preserve the ingredients of organic agricultural material, technologies for this purpose are already developed, among which there are the store in airtight containers (cans); freezing conservation; extraction of chemical compounds and minerals; and drying for grinding (flour). Through an energy balance it would be rather to prefer the product in the form of flour. The flour obtained from the products has the following advantages, drying with renewable energy (solar and wind) at the nopal production sites (dry and semi-dry areas), savings in transportation by the decentralization of the means of processing. There is available technology for the use of the material in the form of flour, the energy consumption is reduced in most cases for flour storage, when compared to other technological possibilities. Likewise, flour would be preferred because it is feasible to produce semi-synthetic foods and perhaps in the near future, synthetic products, based on organic material.

Therefore, it is feasible and profitable to conduct a more comprehensive use of the nopal (*Opuntia* spp.). Likewise, new employment would be generated and a niche of market oriented to efficiently exploit nutrients, previously unused. Hence, it is important to generate technical information, in order to add industrial use to cladodes regardless their age. Among the most useful information needed is the chemical composition and physical properties for post-harvest management that are useful in the elaboration of edible and non-edible products. For this management may begin at the first year of development, continuing onto two or three years old, depending on their attributes for the Cristalina and Peñon Blanco varieties.

Hence the importance of this study, to determine the physical-mechanical characteristics (weight, length, width, thickness, density, internal friction, electrical conductivity) of cladodes, and the flours obtained from them, of nopal varieties Cristalina and Pelon Blanco, one, two and three years of maturity. It is equally important, to supply information for agroindustrial uses. For example, regarding procedures for cleaning, storage, compacting, size reduction, dosing, mixing and screening. As long as cladodes are used in the form of flour to take advantage of the biomass of the mature ones, making them suitable to generate products of agroindustrial interest.

MATERIALS AND METHODS

In this study, freshly cut cladodes (separated from the plant) were used, of two varieties of *Opuntia ficus-indica*, Cristalina and Pelon Blanco, each with one, two and three years of maturity. Likewise, each of them were collected in the autumn season (September-December) and immediately underwent a drying process in an oven (model DZF-6090) at 60 °C for 10 h, in order to dehydrate the samples and proceed to a grinding process to obtain flour. In this process, a Krups GX 410011 model mill was used; concluding with the screening, using a mesh of 0.00381 mm. The values for possible differences in the physical attributes between the varieties of nopal (*Opuntia ficus-indica*) were processed through an analysis of variance, using Statistica v.12 (StatSoft, Inc., Tulsa, OK, USA) in all cases at a significance level $p \leq 0.05$

Methods of measuring physical-technical characteristics

Dimensions

The dimensions of the different cladodes from Cristalina and Pelon Blanco varieties were determined using a common ruler; width, length and thickness were determined (Figure 1). Length (cm) was recorded from the base to the apex, the width (cm) was determined in the widest central area of the cladode and the thickness (cm) was meassured at the thickest zone in the side of the cladode. Measurements were made to 10 cladodes of the Pelon Blanco variety of one, two and three years of maturity; while, for Cristalina variety, seven replicates were determined for each of the years of maturity.

Cladodes Density

To determine the density in the prickly pear cladodes of the Cristalina and Pelon Blanco varieties, a cylindrical container with a capacity of 20 L was used. The procedure was to add water inside up to 3/4 of the total volume, then the water level was marked and a ruler was placed, adhered to the inner perimeter of the container. A cladode was introduced into the container, then the height reached by the water level was marked, this was done to determine the volume of the cladode, then several replicates were made for each of the

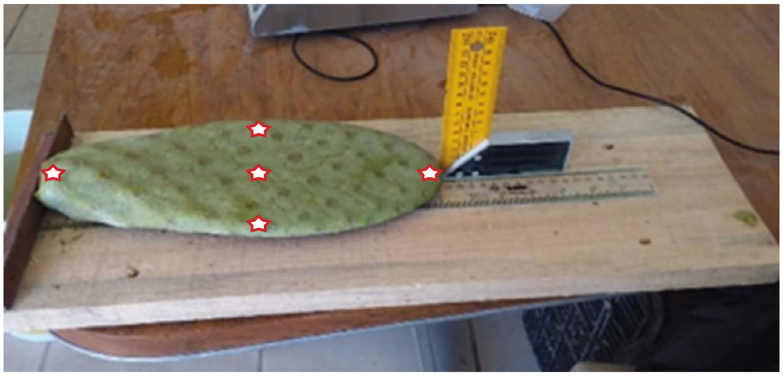

Figure 1. Measurement of the basic dimensions of the prickly-pear cactus (*Opuntia* spp.) cladodes.
☆ Points where measurement of electrical conductivity was done.

three ages of maturity of the cladodes of Cristalina and Pelón blanco varieties. The mass of each cladodes was also recorded with a scale, and the following equation was used to determine the density of the cladodes:

$$\rho = \frac{m}{v}$$

Electrical conductivity

The measurement of the electrical conductivity of the pencas of nopal variety Cristalina and Pelón, were carried out using a digital multimeter (brand Kinzo model 18d265 CE) in five different points on one side of the cladode, performing two replicates of the same points to each cladode and each variety, in the lower section, in the center, at the lateral ends and at the top of the cladode (Figure 1); repeating these same points for each of the six cladodes.

External friction μ_e of the flour from prickly-pear cactus

The materials in contact with the flour of the cladodes were aluminum, ceramics, glass, wood and plastic. With the flour on each of the materials in the form of a plate or thin sheet, each of these was lifted by one of its ends and, at a certain moment, an angle is reached that forms the material with the horizontal, where the flour begins to slide and when it reaches the bottom of the material, the angle formed was recorded with a protractor (Figure 2).

Figure 2. Method for determining the angle of external friction tan $\alpha = \mu_e$

Ten replicate measurements were performed for the flour of each variety on each of the materials.

Internal friction (μ_i) and internal friction angle (α) of *Opuntia* spp. flours

The determination of the internal friction and angle of internal friction was performed to the flours from prickly-pear cladodes of three ages of maturity (1, 2, 3 years) of the two previously dehydrated varieties; 10 replicates were performed for each flour coming from each particular cladode that formed an inverted cone or static triangle (Figure 3).

At this cone, the height and diameter formed were measured. This procedure was repeated 10 times for each of the flours obtained from each prickly-pear variety. The internal fiction and the angle of internal friction were determined using the following equation.

$$\tan \alpha = \mu_i = h/r$$

Where: h=inverted cone height; r=cone base radius.

During the analyses, the variance and comparison of means were determined by the Tukey method ($p \leq 0.05$) of the physical and chemical variables, between cladodes of the varieties Cristalina and Pelón Blanco (*Opuntia* spp.) of one, two and three years of maturity. These analyses were performed in R-project® 4.1.1 under RStudio® 2021.09.0, both freely distributed.

RESULTS AND DISCUSSION

Dimensions

Among the varieties analyzed, significant differences ($p \leq 0.05$) were found only in the width of the cladodes according to the variety; the cladodes of the Cristalina variety were wider than those of Pelon Blanco (Table 1). While the length and thickness did not present a significant difference. According to the analysis of variance, it was not found that age had a significant effect, nor interaction with variety in any of the measured dimensions.

Figure 3. Measurement of the internal friction of the flours.

Table 1. Results of the dimensions in cladodes of prickly-pear cactus, Cristalina, and Pelon Blanco varieties.

Variety	Age (n)	Weight (g)	Length (cm)	Width (cm)	Thickness (cm)	Volume (cm^3)	Density (g/cm^3)
Cristalina	1	1187	39.59 ± 3.44^a	24.61 ± 5.58^a	4.33 ± 0.77^a	1056.83	1.12
Cristalina	2	2550	38.49 ± 9.78^a	24.56 ± 4.64^a	6.81 ± 0.97^a	2642.08	0.97
Cristalina	3	2127	36.73 ± 3.90^a	20.00 ± 3.80^a	8.14 ± 1.69^a	2179.72	0.98
Pelon Blanco	1	1000	37.21 ± 4.47^a	19.17 ± 3.03^b	6.99 ± 9.19^a	936.52	1.07
Pelon Blanco	2	1620	38.37 ± 6.73^a	19.09 ± 1.27^b	5.54 ± 0.73^a	1783.40	0.91
Pelon Blanco	3	1630	40.07 ± 6.68^a	19.09 ± 2.97^b	7.10 ± 1.59^a	1783.40	0.91

Where: Cristalina 7 plants, Pelón Blanco 10 plants. Different letters indicate a significant difference within each column.

Sieving

Results after sieving nopal flours are shown in Table 2.

The decimal errors of the sum result from the rounding of the values and in two cases of the addition, this is due to possible losses. The relationships of values between the years presented the same tendency for Pelon Blanco and Cristalina. Figure 4 shows the behavior of the flour of prickly-pear cactus Cristalina variety where flour particles satisfied the pre-defined standard for wheat, in which between 85 and 90% the particles had a value less than 0.37 mm.

Internal friction of flours

The values of the internal friction of the nopal of different years of maturity are shown in Table 3.

Regarding the external friction of the prickly-pear cactus flours it should be noted that these flour particles of all ages have a size less than 0.59 mm (Figure 4). In addition, that the friction evaluation was performed on flat surfaces of steel sheet wood, glass, vitro-floor, plywood, and plastic, with 10 replicates for each flour on each material. On the other hand, Table 4 highlights that the values of the external friction obtained on the surfaces of the different materials had a value equivalent to that of flours from known grains, such

Table 2. Results of the sieving to define the sizes of the flour particles (100 g grind) of the *Opuntia* spp. varieties under study.

	Grind (g)					
Sieve size	**#30 0.59 mm**	**#80 0.177 mm**	**#100 0.149 mm**	**#400 0.037 mm**	**Fines 0 mm**	**Sum**
Sample	(g)	(g)	(g)	(g)	(g)	(g)
Cristalina 1	27.7	22.8	3.3	42.3	4.5	100.6
Cristalina 2	21.8	27.0	4.3	40.0	2.7	95.8
Cristalina 3	22.0	30.4	4.6	42.6	3.7	103.3
Pelon 1	30.4	23.2	4.4	40.0	2.5	100.5
Pelon 2	27.0	30.5	5.0	37.2	3.6	103.3
Pelon 3	20.0	30.2	5.0	35.6	7.5	98.3

Where: n=(1, 2, 3): age of maturity, years.

Table 3. Results of the internal friction of the flour of *Opuntia* spp. varieties of different age of maturity.

Sample	Age (n)	Average $\mu_i / - /$	S.D. (+/−) $\mu_i / - /$
Cristalina	1	0.962	0.15368077
Cristalina	2	0.911	0.14790763
Cristalina	3	0.959	0.08900062
Pelon blanco	1	0.903	0.13936762
Pelon blanco	2	0.962	0.13685353
Pelon blanco	3	0.969	0.08089087

Where: n=(1, 2,3): age of maturity, years.

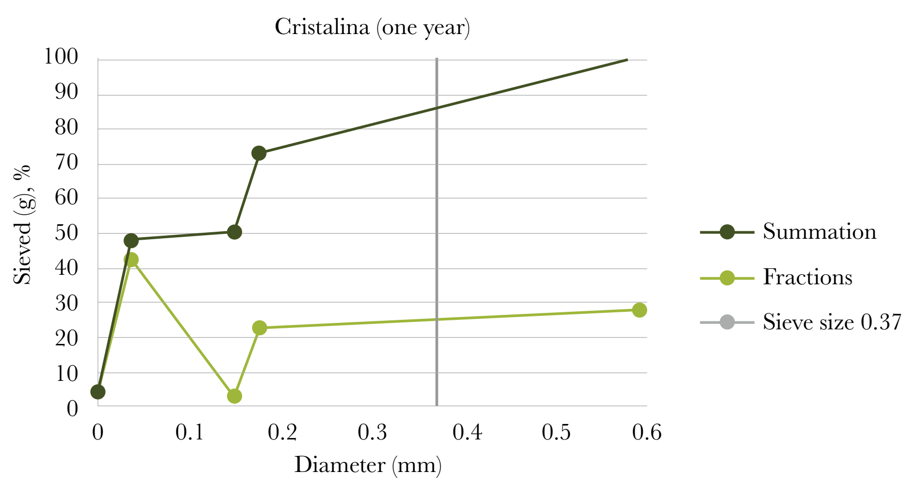

Figure 4. Diameters of the particles of Cristalina flour from cladodes with one year of maturity.

as cereals and maize. Therefore, they can be useful in the food industry and biomaterial generation.

Also, Figure 4 shows that about 80% of nopal flours meet the sizes necessary to achieve an adequate combination with other flours, in order to comply with a quantity of chemical components that may be previously planned for the final product. This means that the values of the external friction in relation to the different materials are suitable for the machines processing those flours selected by the standard size. In addition, flours maintain storage viability for chemical elements with an economic advantage over energy consumption, in regard to other technologies already mentioned. The values and their variation characterize each nopal flour as a technologically manageable flour with the techniques already available.

The values tan α, as equivalents of friction, are important to determine the constructive parameters in different agroindustrial processing; for example, for the transport by gravity in inclined tubes, for the selection of particles by friction when moving them on inclined constructions or moving bands; for the interaction with the surfaces of tools of the machines that divide, mix compact, transport, among others.

Table 4. Results of external friction of flours of *Opuntia* spp. Varieties.

Sample	Material	Average (°)	tan α /-/	Max/Min (°)	Material	Average (°)	tan α /-/	Max/Min (°)
Cristalina 1	Steel sheet	45.3	1.000	56/39	Ceramic	44.5	0.966	52/35
Cristalina 2		41.5	0.869	46/38		41.6	0.869	54/34
Cristalina 3		42.0	0.900	45/37		37.2	0.754	40/32
Pelon blanco 1		39.3	0.810	42/36		42.3	0.900	50/35
Pelon blanco 2		38.3	0.781	41/35		41.4	0.869	49/35
Pelon blanco 3		38.3	0.781	43/34		43.6	0.933	49/34
Cristalina 1	Wood	50.6	1.192	56/45	Playwood	54.3	1.376	59/51
Cristalina 2		54.5	1.376	58/50		53.5	1.327	60/46
Cristalina 3		48.8	1.111	56/45		49.1	1.150	55/44
Pelon blanco 1		51.3	1.235	59/43		54.0	1.376	58/50
Pelon blanco 2		53.7	1.327	61/44		52.5	1.280	58/45
Pelon blanco 3		53.2	1.327	59/49		55.5	1.428	60/50
Cristalina 1	Glass	48.4	1.111	55/39	Plastic	48.5	1.111	58/45
Cristalina 2		46.3	1.035	53/41		53.4	1.327	59/48
Cristalina 3		46.1	1.035	52/36		50.7	1.192	54/46
Pelon blanco 1		42.7	0.900	46/38		50.9	1.192	58/46
Pelon blanco 2		43.9	0.933	55/35		50.0	1.192	60/41
Pelon blanco 3		45.1	1.000	53/36		49.3	1.150	57/41

Where: 1,2 and 3=age of maturity of the cladode, years.

CONCLUSIONS

The physical-mechanical characteristics of the prickly-pear cladodes of different years of maturity can be reliably applied to select the constructive and technological parameters, for machines that reduce particle size.

Equally useful are the values of the internal and external friction of the flours of both prickly-pear varieties, when it is convenient to guarantee a continuous flow during the time of storage, dosing, mixing, among others.

This form of powdered organic material is a modern means of combining different materials, in order to adopt modern forms, from the unification of dust particle sizes. Always within the range of the equivalent standard, for example, corn or wheat, in order to provide a safe storage of renewable minerals. They can also act as a form of input for that technology to be developed in a future towards synthetic materials.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Tricholiperus lipeuroides (Mégnin 1884) in *Mazama temama* (Kerr 1792): first case report in Mexico

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ABSTRACT

Objective: To identify the taxonomy of lice of the family Trichodectidae present in a captive population of *M. temama*.

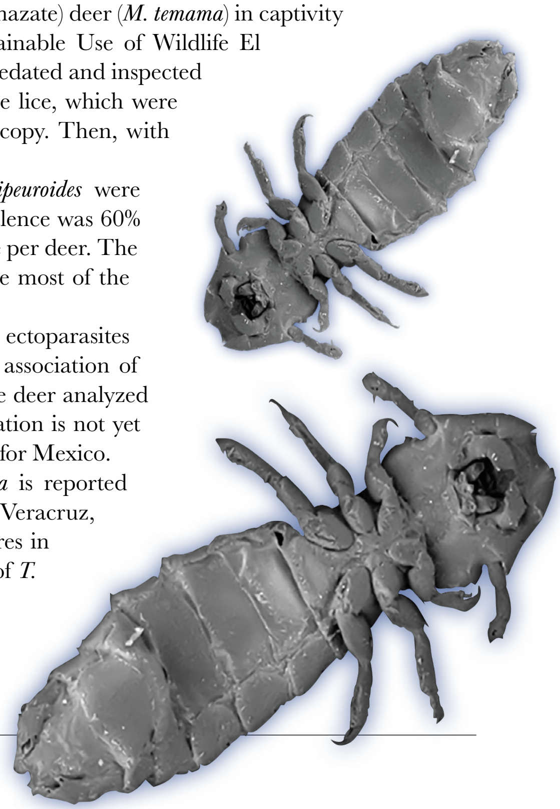
Methodology: Lice were collected from 15 red brocket (Temazate) deer (*M. temama*) in captivity at the Unit for the Conservation, Management and Sustainable Use of Wildlife El Pochote, Ixtaczoquiltan, Veracruz, Mexico. The deer were sedated and inspected in the ventral and inguinal zone, ears and tail to collect the lice, which were observed with bright field microscopy and electron microscopy. Then, with the use of taxonomic keys, lice were identified up to species.

Results: A total of 155 lice identified as *Tricholiperus lipeuroides* were collected in 8 females and 7 males of *M. temama*. The prevalence was 60% (9 out of 15 deer had lice) at an average abundance of 10 lice per deer. The inguinal and ventral zone were the areas of the body where most of the collected lice were detected.

Implications: This study expands the knowledge about ectoparasites in *M. temama* in captivity, recording for the first time the association of the chewing louse *T. lipeuroides* with this species of deer. The deer analyzed did not present clinical signs such as hair loss. This information is not yet reported in other studies; there are no previous case reports for Mexico.

Conclusions: The presence of *T. lipeuroides* in *M. temama* is reported for the first time in the UMA El Pochote, Ixtaczoquitlán, Veracruz, Mexico. Since *M. temama* shares some areas of the enclosures in the UMA with *O. virginianus*, the Temazate became a host of *T. lipeuroides*.

Keywords: ectoparasite, louse, red brocket (Temazate) deer, UMA.



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INTRODUCTION

Chewing lice of the genus *Tricholipeurus*, belonging to the family Trichodectidae, have a paurometabolous life cycle based on three stages: egg, nymph (three stages: N1, N2 and N3) and adult (Mégnin, 1884). These lice measure between 0.8 and 11 mm. Females have an elongated and thin subgenital lobe and are 20% larger than males, which have a reduced head, in the middle part a superficial concave hollow is formed, with thin antennae with three segments and with a claw in each tarsus (Marshall, 1981). Information on the lice-host relationship and the reporting of lice in new hosts (Johnson *et al.*, 2018; Song *et al.*, 2019), as in wild deer (Artiodactyla: Cervidae) or in captivity, is essential information to maintain the health conditions of cervids (Romero-Castañón *et al.*, 2008).

The red Temazate deer, *Mazama temama* (Kerr 1792) is a deer native to the Neotropical region, in which a wide range of pathogens and diseases have been identified (Barbanti *et al.*, 2008). This species is distributed in the central region of Veracruz, Mexico, where environmental conditions prevail for the development of its populations (Ceballos, 2010), such as humid zones and areas of dense vegetation of tropical forests (Serna-Lagunes *et al.*, 2014). However, this species faces conservation problems related to illegal hunting (Salazar-Ortiz *et al.*, 2020), habitat fragmentation (Macario-Cueyactle *et al.*, 2019) and loss of genetic diversity due to geographical isolation (Serna-Lagunes *et al.*, 2021). For this reason, the Management Units for the Conservation and Sustainable Use of Wildlife (UMA) are an *ex situ* strategy for the conservation and management of wild populations of M. temama and their study is important to guarantee the integral health of the species (SEMARNAT, 2008).

Studies on the lice-host relationship in *M. temama* in UMA have been addressed by describing the presence of their endoparasites (Salmorán-Gómez *et al.*, 2019). However, to date, the relationship of the presence of lice of the Trichodectidae family in this species of cervid has not been reported or studied, which is important to guarantee the health of the deer in the UMA. Therefore, the objective of the study was to identify the presence and richness of lice of the family Trichodectidae present in a captive population of *M. temama*.

MATERIALS AND METHODS

In the UMA El Pochote (SEMARNAT code: UMA-IN-CR-0122-VER/og) located in the municipality of Ixtaczoquitlán, Veracruz, Mexico (18° 45' and 18° 57' N; 96° 58' and 97° 06' W). During February to December 2020, seven males (46%, three juveniles and four adults) and eight females (54%; three juveniles and five adults) of *M. temama* were anesthetized. An intramuscular injection of Zelazol® (REG. SAGARPA Q-1196-009) at a dose of 5 mg kg^{-1} (Tilethamine base 50 mg, Zolazepam base 50 mg, and 57.7 mg mannitol per mL) and as an antagonist Tolazoline hydrochloride (2 mg kg^{-1}) intramuscular. It is worth mentioning that the deer were managed under the ethical criteria indicated in the Mexican Standard NOM-051-ZOO-1995 (DOF, 1996) and NOM-062-ZOO-1999 (DOF, 1998).

Each deer was inspected between the hair in the ventral and inguinal area, the ears and the tail, where the detected lice were removed with stainless steel fine-tipped tweezers (Aven®, USA) and placed in polyethylene tubes with 70% ethanol labeled with the collection data. For the taxonomic identification of the lice, these were mounted on slides and brightfield micrographs were obtained with a Carl Zeiss compound microscope, 10X and 40X in the Optical Microscopy Laboratory of the Faculty of Biological and Agricultural Sciences of the Universidad Veracruzana. For the identification of the species, morphological characters were analyzed based on the taxonomic keys of Price and Graham (1997) and Price *et al.* (2003).

In addition, some specimens were selected to obtain micrographs with Environmental Scanning Electron Microscope (ESEM). For this, the samples were mounted on aluminum stubs (filaments) adhered on a double-sided carbon conductive tape and a retro-dispersed electron detector in 20 kV and 20 Pa of pressure. The heels were directly observed, and the images were captured at a magnification 200X and 1000X in a Carl Microscope Zeiss model EVO LS10 (Munich, Germany) and stored in TIFF format (2048×1536 px). The Laser Scanning Confocal Microscopy (CLSM) technique was also performed; the samples were mounted on glass slides to be observed in lambda mode in parallel. A sequence of images was obtained at laser wavelengths of 405 nm, 488 nm, 561 nm, and 640 nm (4% capacity) in a Carl Zeiss A microscope model LSM 800 (Munich, Germany) (Delgado-Núñez *et al.*, 2020).

The images were taken at 20X and 40X magnification with the Apochromatic Plan, 1.3 numerical aperture and 1 Airy Unit (AU) of pinhole aperture at a 2048×2048 px resolution in TIFF format, using a coupled HD camera (AxioCam, Carl Zeiss, Model 305, color, Göttingen, Germany). The images were handled using the ZEN 2.6 Zeiss Blue editing software (Delgado-Núñez *et al.*, 2020). Frequency analyses were performed to describe the sex ratio of the collected lice, and to describe the proportion of lice collected regarding the sex and age category of the sampled deer.

RESULTS AND DISCUSSION

The lice collected in *Mazama temama* were identified as *Tricholipeurus lipeuroides* (Mégnin, 1884). The lice presented morphological structures that coincided with the characteristics described in the taxonomic key such as the head wider than the prothorax, the jaw under the head, a nail in each tarsus, filiform antennae and divided into three segments. In the males, also it was observed an enlarged basal segment (Figure 1).

The morphological identification structures obtained by electron microscopy (Figure 2), allowed to observe details of the shape, texture, and surface of the lice such as the jaw, silks (hairs), legs with claws, and reproductive organs. These characteristics corresponded to the taxonomic identification of the species of chewing louse *T. lipeuroides*, a new ectoparasite recorded in the red brocket (Temazate) deer, *M. temama*.

A total of 155 chewing lice were collected just in the groin and ventral zone of *M. temama*. Out of the 15 *M. temama* deer evaluated, only nine presented lice, this is equivalent to a prevalence of 60%. Of those nine deer with lice, five males had 105 lice equivalent to 68%, and four females 50 lice, which corresponded to 32%. According to the age category of deer, adults had a higher proportion of lice 77%, compared to juvenile deer, 33%.

This study reports for the first time the presence of the chewing louse *T. lipeuroides* in *M. temama* in captivity conditions in Veracruz, Mexico, as *M. temama* has not been reported in

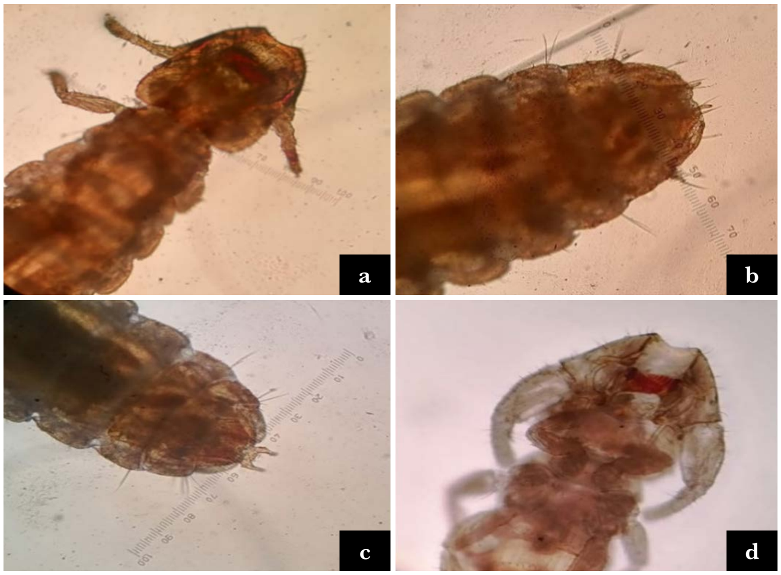

Figure 1. *Tricholipeurus lipeuroides* female: a) head; (b) abdomen; male: c) abdomen; (d) head.

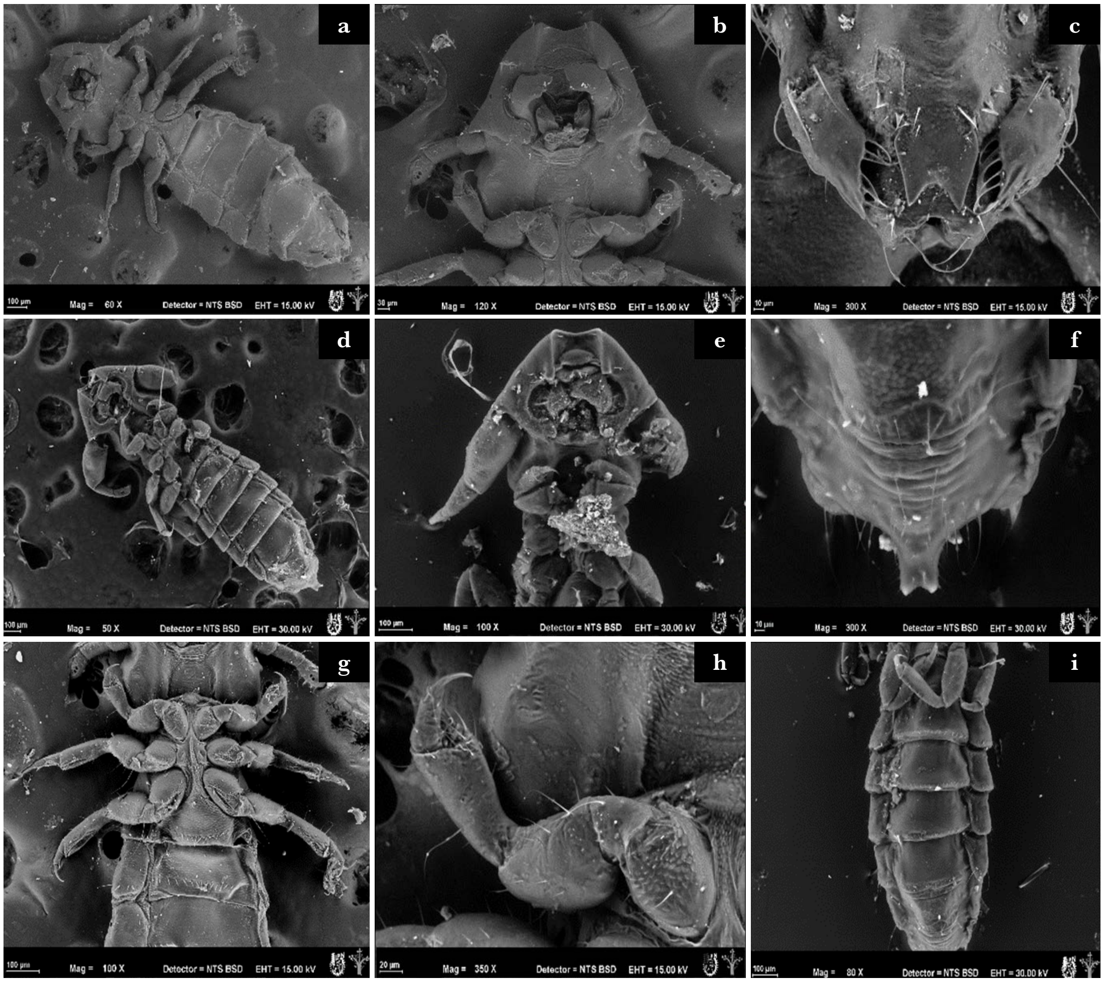

Figure 2. Female: a) ventral view, b) head-jaw, c) genitalia; male: d) ventral view, e) head-antennae, f) genitalia, g) thorax, h) leg-nail, i) abdominal segments.

other studies as a new host of *T. lipeuroides*. Due to the conditions of management, control of parasites and diseases, and the movement of deer to different management pens within the same UMA El Pochote, this could promote exchanges of *T. lipeuroides* to *M. temama* (Salmorán-Gómez *et al.*, 2019).

In other countries, the presence of *T. lipeuroides* has been reported in white-tailed deer fawns (*O. virginianus*) in Canada (Colwell *et al.*, 2008) and in four white-tailed deer in the Virgin Islands National Park, USA (Mertins *et al.*, 2017). However, for Mexico, the first report of *T. lipeuroides* was in Mexican white-tailed deer (*O. virginianus mexicanus*; Sánchez-Montes *et al.*, 2018) and for Veracruz white-tailed deer (*O. virginianus veraecrucis*; Estrada-Souza *et al.*, 2020), but this is the first case report of *T. lipeuroides* in *M. temama* for Mexico.

In other studies, with black-tailed deer (*O. hemionus columbianus*) it was found that some species of chewing lice can affect the health of deer when the diet does not meet their nutritional needs. Because the immune system does not act properly and causes diseases such as hair loss syndrome (Foreyt *et al.*, 2004). However, in the Temazate deer evaluated in this study, those signs were not present; thus, a constant monitoring of the integral health of the temazates would guarantee the viable management of the population in the UMA El Pochote.

The chewing louse *T. lipeuroides* has been considered an ectoparasite specific to its host (Strickland *et al.*, 1981). However, factors that influence presence such as age and sex of the deer, should be evaluated in future studies to determine the parasite-host relationship (Forrester *et al.*, 1996).

CONCLUSIONS

The presence of *Tricholipeurus lipeuroides* in *Mazama temama* is reported for the first time to our knowledge at the UMA El Pochote, Ixtaczoquitlán, Veracruz, Mexico. Although chewing lice are present in red brocket (Temazate) deer, these latter do not present any clinical signs of disease such as hair loss. It is possible that by sharing some areas of the enclosures and management pens of the UMA El Pochote with *O. virginianus*, the Temazate deer became an incidental host of *T. lipeuroides*; sometimes in the UMA El Pochote other deer are received by exchange, donation or seizing. The greater number of lice was found in males of Temazate deer than in females. This finding suggests implementing studies on the influence of sex, age, and reproductive condition of the host on the presence of chewing lice. Since the gravid females of *M. temama* that were evaluated in this study did not present lice.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Spent mushroom compost for germination and growth of *Ricinus communis* L.

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ABSTRACT

Objective: To determine the response of castor oil seeds and seedlings to six different substrata: regional soil (sandy-clay-loam), sand, spent mushroom compost (CAC), and mixes of CAC with 50% (A1:CAC1), 66% (A1:CAC2), and 33% (A2:CAC1) sand.

Design/Methodology/Approach: The experimental design consisted of two randomized complete blocks with 24 experimental units per substratum, monitored from their germination until 50 days later. Seed weight was correlated with the final seedling biomass.

Results: The best germination ($P<0.05$) was obtained with the A2:CAC1 and A1:CAC1 substrata, while regional soil had the lowest germination. CAC produced the highest growth and biomass in 50-day-old plants ($P<0.05$), followed by A1:CAC2, A1:CAC1, and A2:CAC1, while regional soil and sand had the lowest development. Seed weight had a statistically positive correlation ($P<0.05$) with seed biomass only in the sandy substratum.

Study Limitations/Implications: The experimental period and pod size limited the achievements of this research.

Findings/Conclusions: Independently of seed weight, CAC, and mixes with sand provide suitable texture and nutrient contents for castor oil seedling's germination and growth.

Keywords: seeds, seedlings, substrata, soil type.

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INTRODUCTION

The castor oil plant, or Ricinus (*Ricinus communis* L.), is an oily species with vast industrial applications, such as oils, lubricants, cosmetics, biofuels, and pharmacological products (Yeboah *et al.*, 2021). In Mexico, its cultivation was limited to only 1,044 ha in the entire territory in 2020 (SIAP, 2021); consequently, more than 6.6 million US dollars of castor oil was imported for industrial uses in the center of the country (DataMéxico, 2021). Therefore, its cultivation can generate significant income in the primary sector. An essential factor for its establishment in the places where it is intended to be cultivated is to determine its growing characteristics and its agricultural demands. In this sense, animal- and vegetable-waste-based composts are good sources of nutrients and contribute

Image by Alejandro Sánchez

to the sustainable improvement of soil structure since its nutrients are reintegrated into the system, avoiding their contribution to landfills (Adugna, 2016).

Spent mushroom compost (CAC) is the residual product of mushroom (*Agaricus bisporus* Lange) production. Although useless for the mushroom, it still has high levels of nutrients and enzymes (García-Mendívil *et al.*, 2014b). The agricultural use of CAC has been shown to improve the production of beans (*Phaseolus vulgaris* L.) and wheat (*Triticum* spp.) by increasing their productivity (García-Mendívil *et al.*, 2014a, 2014b). However, its use has not yet been tested in *R. communis*. In addition, *Ricinus* has not been commercially established yet in the central region of the state of Veracruz; consequently, its response to the site's soils is unknown.

Additionally, suitable substrata for its germination and growth have not been evaluated. Previous works have analyzed the germination and growth of castor oil plants in different substrata, such as sand, mixtures of sand with tezontle and compost (type of compost not specified), and regional soils. However, the substrate has never been evaluated, only plant provenances (Solís-Bonilla *et al.*, 2011, 2017; García-Herrera *et al.*, 2019). Meanwhile, although the importance of seed weight for better plant growth is known (Valdés-Rodríguez *et al.*, 2018), the response of seed biomass of *R. communis* to different substrata has not yet been researched. Therefore, further research is required to generate adequate recommendations for producers interested in venturing into this crop. Therefore, this research aimed to evaluate the response of *R. communis* seeds and seedlings to germination and development in the regional soil, sand, and sand-based mixtures and spent mushroom compost.

MATERIALS AND METHODS

Experimental site and plant material

The study was carried out in the city of Xalapa, Veracruz, Mexico (19° 30' 54" N and 96° 55' 05" W). It has a semi-warm humid climate, with average temperatures between 18 and 20 °C (INEGI, 2018). Seeds of *R. communis* were collected from a mother plant from the state of San Luis Potosí, in the Mexican Plateau (22° 36' 12" N, 100° 24' 47" W). The plants had the following characteristics: medium-sized plants (2-3 m), serrated purple leaves, glands on the margin, reddish brown veins, and red petioles; purple flower clusters with medium-sized fruits (2.5-3.0 cm) and greyish-purple seeds. Each seed was weighed individually with an analytical balance (Ohaus 310 g*0.1 g), obtaining an average weight of 491 mg per seed ± a standard deviation of 44 mg per seed. The following substrata were used: coarse sand (A) (1-2 mm) from the region, regional soil (soil extracted from the surface up to a depth of 30 cm) and spent mushroom compost (CAC). Additionally, the following ratio combinations of sand and compost were used: A1:CAC1 (50:50), A1:CAC2 (33:66), and A2:CAC1 (66:33). The silica sand was obtained in the suburbs of the municipality of Xalapa, Veracruz, Mexico. The CAC came from Altex Rioxal, a company that operates in the town of Las Vigas, Veracruz, Mexico, and which uses wheat straw enriched with wheat bran, gypsum as a structure improver, and sodium carbonate as an acidity or alkalinity stabilizer.

Experimental design and measuring

The experimental design consisted of a randomized complete block with 24 experimental units per substratum and two repetitions. The experiment was established in a 5 m×15 m×2 m (width×length×height) greenhouse covered with polyethylene. One seed per 15×20 cm black plastic bag was sown in their corresponding substratum. The first repetition (Rep1) began on November 27, 2014, and the second (Rep2) started on January 5, 2015. The number of sprouted seedlings was recorded daily. For each treatment, the germination percentage (the number of emerged plants divided by the number of seeds sown) and the mean germination time by substratum (the average number of days it took for all the sprouted seedlings) were estimated. The seedlings were watered every three days until the substratum was saturated. For each plant, the following variables were recorded every four days: plant height (metric tape), root collar diameter (digital vernier) at the base where it emerged, length and width of the largest leaf, and the number of leaves. The leaf area was estimated using the following equation: Area=0.55×length×width of the leaf (Jain & Misra, 1966). The observation period per plant was 50 days. Each plant was removed at 50 days, and the length and diameter of the taproot and primary lateral roots were measured with a measuring tape and a digital vernier, respectively. Also, the number of primary lateral roots (those sprouting directly from the stem base) was counted. Subsequently, the plant was separated into roots, stem, and leaves to obtain the fresh and dry weight of each one with an Ohaus digital scale (310 g*0.1g). During the experiment, the maximum and minimum temperatures and the environmental humidity were recorded daily with a digital thermo-hygrometer.

Substratum analysis

The pure substrata (sand, soil, and compost) were analyzed to determine texture (Bouyoucos method), pH (in aqueous solution, digital potentiometer), electrical conductivity (CE, conductivity meter), and organic matter (Walkley and Black). The textures of the substrata were divided as follows: sand (sandy), regional soil (sandy-clay-loam), and compost (sandy-loam-silty) (USDA, 2022) (Table 1). Organic matter was considered very low for sand, medium for soil, and high for CAC. The electrical conductivity had negligible salinity effects in the sand and regional soil, but it was slightly saline for CAC. The pH was considered neutral in the three substrata (SEMARNAT, 2002).

Table 1. Chemical analysis of the base substrata used to germinate *Ricinus communis* seeds.

Substratum	Textural composition (%)	Organic matter (%)	Electric conductivity (dS m^{-1})	pH
Sand	Sand: 91.3, clay: 0, silt: 8.7	0.32	0.04	7.10
Regional Soil	Sand: 59.3, clay: 22.4, silt: 18. 3	3.12	0.97	6.65
CAC	Sand: 62.6, clay: 16.0, silt: 21.4	27.17	2.83	6.85

CAC: spent mushroom compost.

Growth dynamics

The growth dynamics of the height, root collar diameter, leaf area, and the number of leaves were evaluated using a parameterized Gompertz model (Mora-Chacón *et al.*, 2022), represented by Equation 1.

$$V(d) = Is * \frac{e^{-e\beta_1(d_b - \beta_2)}}{e^{-e\beta_1(d - \beta_2)}} \tag{1}$$

Where: $V(d)$ is the value at time d, Is is the average value of the series at the base time, d is the time at which the function is evaluated, d_b is the base period, and -1 and -2 are constants that model the growth and shape of the curve. The increase in leaf area was represented by the straight-line equation (*area=slope*time+constant*) because this model obtained a higher coefficient of determination than the Gompertz model. The coefficient of determination (r^2) was obtained with the least squares method to determine the representation of each variable.

Statistical analysis

The Log Rank test ($P \leq 0.05$) was used to evaluate germination and survival between treatments. This test is considered appropriate to analyze events that change over time with probability distributions of the germination curve type (Romano and Stevanato, 2020). Final biomass and morphological variables were subject to an analysis of variance for a randomized complete blocks model with two replicates, using substratum and replicates as factors. In all cases, mean comparisons were performed using Tukey's test ($P \leq 0.05$). Correlations between seed weight and the final seedling biomass were performed using a Pearson linear correlation for each substratum. The SigmaPlot 10.0 software was used for all tests.

RESULTS AND DISCUSSION

Germination and survival

The Log Rank test showed significant differences between treatments (Rep1, $P<0.001$; Rep2, $P=0.046$). The sand and compost substrata (A1:CAC1 and A2:CAC1) obtained the highest germination rates and the shortest germination times, while the sandy-clay-loam soil recorded the lowest and slowest germination (Table 2). Consequently, sand-compost mixtures were the most suitable substrata for germination because they were light and nutritious, with a pH close to neutral. Since the CAC substratum was treated against mushroom pathogens, the mixtures allowed good aeration, stimulated germination, and prevented diseases (Sun *et al.*, 2013; García-Mendívil *et al.*, 2014). Improvements in germination with substrata that combine sand and compost are also reported for other oily seeds, such as *Jatropha curcas* (Díaz-Chuquizuta *et al.*, 2017), highlighting the ability of the compost to degrade seed coat and improve the imbibition of the seed. The average germination times (Table 2) were like those reported by Valdés-Rodríguez and Pérez-Vázquez (2019) at 22-28 °C temperatures, 71-89% humidity levels, and 4-10 days

Table 2. Average environmental conditions during germination of *R. communis* in different substrata.

Substratum and environmental conditions	Germination (%)		Mean germination time (days)	
	Rep1	Rep2	Rep1	Rep2
Sand	95.8 b	91.7 b	8.9	9.2
Regional soil	66.7 c	91.7 b	9.6	9.3
CAC	91.7 b	91.7 b	9.3	9.3
A1:CAC2	100.0 a	95.8 ab	7.2	9.0
A1:CAC1	100.0 a	100.0 a	7.8	8.9
A2:CAC1	100.0 a	100.0 a	7.8	9.0
Maximun temperature (°C)	26.9	23.7		
Minimun temperature (°C)	19.8	15.9		
Humedad ambiental (%)	80.1	79.6		

Rep1: November 27, 2014; Rep2: January 5, 2015. CAC: spent mushroom compost, A1:CAC1: 50 % sand y 50 % CAC, A2:CAC1: 66 % sand y 33 % CAC, A1:CAC2: 33 % sand y 66 % CAC. Different letters in the same column indicate statistical differences (Tukey, $P \leq 0.05$).

on average. In this regard, *R. communis*' great plasticity allows it to adapt to different temperatures but germinates better at ranges from 20 to 30 °C (Ribeiro *et al.*, 2014). In this work, the minimum temperatures recorded in Rep2 (3.9 °C less than in Rep1) could have caused a slight delay in the mean germination time, which on average, lasted 1.4 days more in mixtures with compost.

Plant growth

The analysis of variance showed significant differences between treatments for all variables and between repetitions for plant height and leaf area ($P<0.001$). The CAC substratum recorded the largest sizes in all the variables, followed by the A1:CAC2 substratum, which was similar to the CAC in plant height and number of leaves (Table 3). The sand substratum showed the lowest growth, while height growth and diameter thickness increased by 38% and 18%, respectively, in the region's soil compared to the sand substratum. The minimum addition of CAC (A2:CAC1) resulted in significant increases in all variables, with plant height values 2.2 times higher, stems 1.4 times thicker, and 3.3 times greater leaf area than in the sandy substratum. Leaf area and stem thickness showed the most significant growth responses to increments in the percentage of CAC. These results indicate that the CAC substratum or its mixtures with sand provide the nutrients, the pH, and a suitable texture to achieve better seedling development. In this regard, the texture and pH of the CAC were ideal for the growth of *R. communis* since the roots of this species are better suited to penetrate light-texture soils with 6-7 pH levels, which facilitates nutrient absorption (Souza *et al.*, 2014; Wang *et al.*, 2019). Concerning the alkalinity of the CAC, Soares de Lima *et al.* (2015) found that *R. communis* grows well with CE values of 2.1 dS m^{-1} and the results in this work confirm that slightly higher levels do not affect plant development. The environmental conditions explain the differences between repetitions: in Rep1, the average temperature was 16.1 °C, while Rep2 recorded 16.9 °C and 38 more minutes of sun exposure. These conditions favor a more significant development of the stem

Table 3. *R. communis* plant measurements in different substrata 50 days after germination.

Substratum	Root collar diameter (mm)	Plant height (cm)	Number of leaves	Leaf area (cm^2)
Sand	5.29 e	10.04 d	5.00 c	21.10 c
Regional soil	6.59 d	16.54 c	5.47 bc	50.59 d
A2:CAC1	7.26 c	22.33 b	5.81 ab	69.84 c
A1:CAC1	7.44 bc	22.46 b	6.12 ab	84.82 b
A1:CAC2	7.77 b	25.44 ab	6.00 ab	96.04 b
CAC	8.55 a	26.09 a	6.19 a	116.53 a

CAC: spent mushroom compost, A1:CAC1: 50% sand y 50% CAC, A2:CAC1: 66% sand y 33% CAC, A1:-CAC2: 33% sand y 66% CAC. Different letters in the same column indicate statistical differences (Tukey, P≤0.05).

and leaf area since the growth response of *R. communis* increases in higher temperatures and solar radiation due to its high photosynthetic capacity (Ribeiro *et al*., 2014).

Concerning the growth dynamics (Figure 1), the Gompertz model explained 95 to 99% of the average variation in heights and number of leaves and 85 to 96% in root

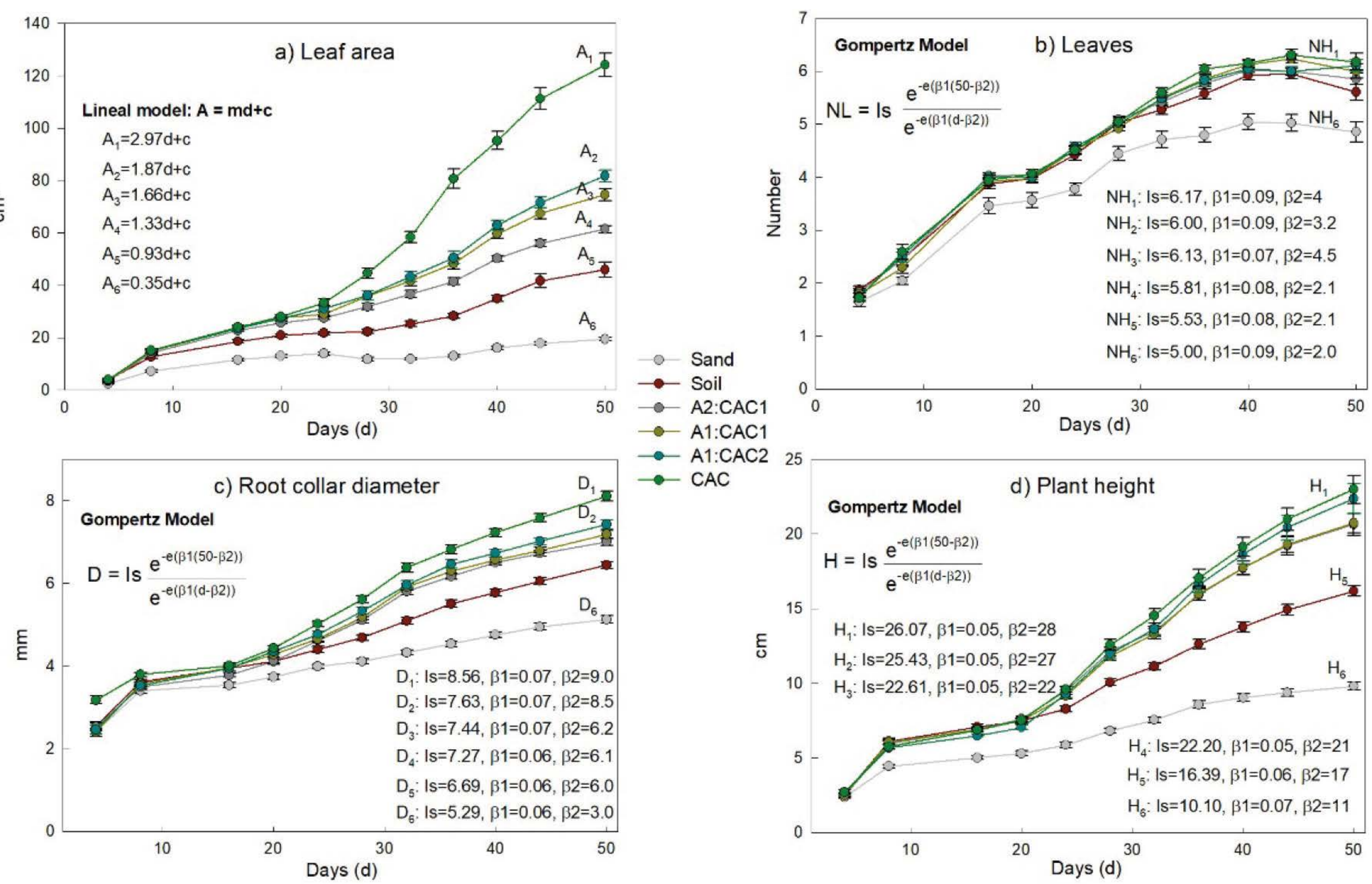

Figure 1. Aerial growth of *Ricinus communis* on different substrata. Each point represents the mean of 48 replicates ± standard error. A: leaf area, NH: number of leaves, D: root collar diameter, AP: plant height, CAC: spent mushroom compost, A1:CAC1: 50% sand and 50% CAC, A2:CAC1: 66% sand and 33% CAC, A1:CAC2: 33% sand and 66% CAC.

collar diameter. Therefore, this model is considered appropriate to represent the growth dynamics of *R. communis* seedlings (Mora-Chacón *et al.*, 2022). The -2 coefficient —related to the growth rate of the curve— indicates an increase of up to 2.5 times in seedling stems in CAC compared to those in the sand substratum. Regarding the leaf area, linear growth had rates eight times higher in CAC than in sand. These increases in foliage growth rates are mainly attributed to the high content and availability of N that CAC possesses, which allows an accelerated development of seedlings, especially in the foliar area (Nahar and Pan, 2015). On the other hand, the progressive decrease in growth (*e.g.*, the number of leaves) can be explained by the size of the container bags that limited the development of the plants because this depends on the size of the pod (SEB, 2012).

Root systems

The analysis of variance showed statistical differences between substrata in all variables but only found differences between repetitions in the number of roots, with an average of only 4% more lateral roots in Rep2 than in Rep1 (Table 4). The CAC substratum recorded the thickest taproots, while the total length and diameter of lateral roots were similar in all treatments with some CAC ratio but were higher than those obtained in soil and sand. The light textural composition and nutrient concentration in CAC resulted in thicker roots, allowing good aeration and facilitating their development in length and thickness (Nahar and Pan, 2015). Nevertheless, roots are usually thinner in a nutrient-poor substrate (Helliwell *et al.*, 2019), as can be seen in the case of the roots in the sand, which were thinner than in substrata with some compost ratio.

Seedlings fresh and dry weight

The analysis of the variance of fresh weights found highly significant differences between the substrata and the repetitions in all plant parts ($P<0.001$). Highly significant differences were also found in plant dry weight, although there were no differences in the stem between repetitions (Table 5). The fresh and dry weights of stems, leaves, and roots were statistically higher in the CAC than in the other substrata. A1:CAC2 and A1:CAC1

Cuadro 4. Average root measurements of 50-day-old *R. communis* seedlings in different substrata.

Substratum	Taproot length (cm)	Lateral roots length (cm)	Taproot diameter (mm)	Lateral roots diameter (mm)	Number of lateral roots
Sand	31.80 c	31.14 c	3.38 d	0.87 b	32.77 a
Suelo de la región	30.03 c	30.53 c	3.95 c	0.87 b	34.85 a
A2:CAC1	34.28 bc	33.52 bc	4.78 b	1.00 a	34.33 a
A1:CAC1	37.07 ab	34.74 ab	5.05 b	1.04 a	35.18 a
A1:CAC2	37.18 ab	35.46 ab	5.22 b	1.09 a	33.35 a
CAC	39.77 a	36.91 a	6.09 a	1.10 a	34.73 a

CAC: spent mushroom compost, A1:CAC1: 50% sand y 50% CAC, A2:CAC1: 66% sand y 33% CAC, A1:CAC2: 33% sand y 66% CAC. Different letters in the same column indicate statistical differences (Tukey, $P\leq 0.05$).

Cuadro 5. Average fresh and dry weights of *R. communis* in different substrata 50 days after germination.

Substratum	Fresh weight (g)			Dry weight (g)		
	Leaves	Stem	Roots	Leaves	Stem	Roots
Sand	1695.50 f	1601.5 e	4242.9 e	307.29 e	271.88 e	428.96 e
Regional soil	3902.30 e	4090.7 d	5916.4 d	646.15 d	555.29 d	559.02 d
A2:CAC1	5371.50 d	7255.6 c	11187.1 c	819.85 c	767.00 c	787.42 c
A1:CAC1	6545.30 c	8206.0 bc	12448.6 b	1024.35 b	888.27 b	877.60 b
A1:CAC2	8319.30 b	8834.3 b	12814.0 b	1067.73 b	898.79 b	900.94 b
CAC	11387.10 a	11396.5 a	16001.8 a	1660.60 a	1189.92 a	1160.59 a

CAC: spent mushroom compost, A1:CAC1: 50% sand y 50% CAC, A2:CAC1: 66% sand y 33% CAC, A1:CAC2: 33% sand y 66% CAC. Different letters in the same column indicate statistical differences (Tukey, $P \leq 0.05$).

recorded similar weights, and they outperformed A2:CAC1, which was superior to the plants in regional soil, while they were more developed than the ones in the sand. This behavior implies that the higher the nutrient concentration in the substratum, the greater the development of all the plant organs. In this regard, studies with CAC and beans (*Phaseolus vulgaris* L.) showed that compost increases the photosynthetic capacity of plants (García Mendívil *et al*., 2014), enhancing their ability to assimilate and process nutrients. For their part, Nahar and Pan (2015) found that the response of 28-day-old seedlings of *R. communis* to different nitrogen levels is directly proportional to the increase in their biomass, indicating that high levels of nitrogen favor the initial growth of *R. communis.* It is also noteworthy that the root-biomass ratio increased in the substrata with fewer nutrients: 31% in compost and 43% in the sand. This performance indicates that the roots of the plants in substrata with few nutrients had a more significant development than their foliage, which allowed them to explore their medium better and obtain more nutrients (Azcón-Bieto and Talón, 2013).

Correlation of seed weight with final dry weight per substratum

The correlations between seed weight and seedlings' dry weight show that seed weight only has a positive and significant correlation ($P \leq 0.05$) with plant biomass in the sandy substratum (the least nutritious substratum). Meanwhile, there is only a positive correlation, but not significant, between the regional soil and the substrata with the lowest compost content (Table 6). These values indicate that nutrient content in CAC and their mixes produce such development in the plants that seed biomass does not play a predominant role in the growth of the *R. communis* seedlings. Meanwhile, seed weight is essential in the less nutritious substratum to obtain additional energy for the plant's better development and survival under unfavorable conditions (Kolodziejek, 2017). These results also match the findings of Valdés-Rodríguez and Pérez-Vázquez (2018), who found significant positive correlations between *R. communis* seedlings biomass and their seed weight in a sandy and nutrient-poor substratum 59 days after germination.

Table 6. Correlation coefficients between seed weight and final dry weight of *R. communis* seedlings at 50 days.

Substratum	Leaf	Stem	Root
Sand	0.56*	0.65*	0.57*
soil of the region	0.18	0.27	0.37
A2:CAC1	0.21	0.32	0.29
A1:CAC1	0.00	0.10	0.05
A1:CAC2	0.07	0.28	0.06
CAC	−0.31	−0.35	−0.31

*Significative to $P \leq 0.05$.

CONCLUSIONS

R. communis seeds can germinate in less time and with a higher percentage in light substrata mixed with spent mushroom compost (CAC) and sand. When CAC is the only element of the substratum, it enables the best development of stems, leaves, and roots compared to its mixtures with sand in ratios of up to 66%. Besides, root and aerial development of *R. communis* seedlings is significantly improved in soils with up to 33% CAC mixed with sand compared with sandy substrata with a low content of organic matter or a sandy-clay-loam soil moderately rich in organic matter. Seed weights positively impacts the growth of *R. communis* seedlings only in nutrient-poor sandy substrata, but its effect cannot be determined in medium- or high-nutrient substrata.

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AGRO PRODUCTIVIDAD
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In vitro biogas production and degradations of sheep diets containing *Crotalaria* or Chipile at two different regrowth ages

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ABSTRACT

Objective: To determine the *in vitro* biogas production and fermentative characteristics of diets for fattening lambs containing 20% chipile or crotalaria at 30 or 40 d of regrowth.

Methodology: The treatments were whole diets containing 20% crotalaria with 30 d (T1) or 40 d of regrowth (T2), as well as 20% chipile with 30 d (T3) or 40 d of regrowth (T4). *In vitro* gas production was measured at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h and the following elements were determined: kinetic estimators (A, b, k), dry matter (DMD), organic matter (OMD), neutral detergent fiber (NDFD), and acid detergent fiber (ADFD) degradation, metabolizable energy (ME), and short-chain fatty acids (SCFA). The experimental design was completely randomized.

Results: Regarding the accumulated biogas production, T4 presented higher production from 2 to 24 h, T3 and T4 higher at 48 h, and T1 higher at 72 h. Kinetic estimators showed that T1 was higher in A and k and T4 was higher in b. T2 presented the lowest DMD, OMD, NDFD, ADFD, ME, and SCFA.

Study Limitations: Scale production of chipile, aimed to obtain a greater biomass volume, is limited since it has not been domesticated yet.

Conclusions: Sheep diets containing 20% chipile or crotalaria with 30 d of regrowth have proven to be an alternative for the manufacturing of whole diets for the intensive fattening of lambs in the tropics.

Keywords: Biogas, degradation, metabolizable energy, alternative feed.

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INTRODUCTION

Stock breeding, supplier of food and raw materials of animal origin, constitutes one of the main economic activities of the country's primary sector. In Mexico, the inventory of sheep production is 8,725,882 heads, and the State of Mexico (15.8%) and Hidalgo (12.9%) have the largest inventory, while the state of Guerrero only has a 2% stake (SIAP, 2021). Sheep production in Mexico is carried out under traditional grazing systems, with little technology and low productivity. Meat production is the most widespread productive

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activity in rural areas, since it is carried out even under adverse weather conditions that hinder other productive activities. However, there have been significant changes in recent years because it is no longer a backyard activity, a forced savings activity, or an activity that makes marginal use of summer pastures (Hernández-Marín, 2018).

In the animal production systems of the tropical zones of Mexico, feeding is based on grazing. However, the nutritional quality is not enough to cover the animals' nutritional requirements; therefore, the use of legumes is an alternative in animal feed (Gámez *et al.*, 2019). There are between 16,000 and 19,000 species of legumes distributed all over the world. They are divided into 750 genera and characterized by their high protein content (20 to 45%) (Benites, 2020). The Crotalaria genus comprises about 600 species that are distributed throughout the tropical and subtropical regions of the world. Chipile (*Crotalaria longirostrata*), native to Mesoamerica, is a legume of the Fabaceae family. It is a wild plant that grows in Chiapas, Mexico, and is traditionally used for human consumption and as forage for animals (Miranda-Granados *et al.*, 2018; Córdova-Ballona *et al.*, 2022). For its part, crotalaria (*Crotalaria juncea*) is a legume with high biomass production; it is native to India and is used as forage, green manure, and biological agent (Avendaño, 2011); due to its phenolic content, part of the seed has a significant antibacterial activity (Chouhan and Singh, 2010). Crotalaria foliage can be used as a high-quality and palatable forage source during the vegetative phase. Approximately six to eight weeks after planting or cutting, it has a high concentration of nitrogen and protein (18-22%), phosphorus (0.29%), and calcium (1.4%), as well as 60% digestibility (Gámez *et al.*, 2019). The hypothesis was that the use of chipile with 30 and 40 d of regrowth will achieve better biogas production values and in vitro fermentative characteristics in diets for fattening lambs than crotalaria with the same number of regrowth days. Therefore, the objective was to determine the *in vitro* biogas production and fermentative characteristics of diets for fattening lambs containing 20% chipile (*Crotalaria longirostrata* Hook. & Arn) or crotalaria (*Crotalaria juncea* L.) with 30 or 40 d of regrowth.

MATERIALS AND METHODS

Study site

This work was carried out in the Laboratorio de Nutrición Animal of the Facultad de Medicina Veterinaria y Zootecnia No. 2 of the Universidad Autónoma de Guerrero, located at km 197 of the Acapulco-Pinotepa Nacional highway, Cuajinicuilapa, Guerrero, Mexico. It is located at 16° 28' 28" N and 98° 25' 11.27" W, at 46 m.a.s.l.

Chemical analysis

Chipile and crotalaria samples (Table 1) were dehydrated at 60 °C for 72 h in an oven (Riossa HCF-41, Mexico). Subsequently, they were ground using a 1mm screen in a Thomas-Wiley mill (Thomas Scientific, Swedesboro, NJ, USA). Dry matter (DM), crude protein (CP), and ash (As) were determined in the samples according to the AOAC methods (2005) no. 930.15, 920.105, and 942.05, respectively. Organic matter (OM) was estimated by subtracting the ash value from 100. In addition, neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined using the ANKOM Technology Method according

Table 1. Chemical composition of crotalaria and chipile with 30 and 40 d of regrowth.

	Crotalaria		**Chipile**	
	30 d	**40 d**	**30 d**	**40 d**
Dry matter (%)	18.23	21.86	16.29	12.33
Crude protein (%)	15.86	10.37	25.82	24.71
Neutral detergent fiber (%)	58.85	68.84	55.16	48.79
Acid detergent fiber (%)	39.54	52.89	34.47	32.23
Hemicellulose (%)	19.31	15.95	20.69	16.57
Organic matter (%)	90.91	94.63	91.75	92.56
Ash (%)	9.09	5.37	8.25	7.44

to Van Soest *et al.* (1991). Hemicellulose was calculated by the difference between NDF and ADF.

Treatments

The treatments were whole diets for fattening lambs (Table 2) containing chipile (*Crotalaria longirostrata*) or crotalaria (*Crotalaria juncea*) with 30 or 40 d of regrowth (Table 2).

***In vitro* test**

The medium components contained 30 mL of clarified rumen fluid [fresh bovine rumen fluid centrifuged for 10 min at 12,857 *x g* and sterilized (All American® 1941X,

Table 2. Composition of the treatments that include 20% chipile or crotalaria with 30 or 40 days of regrowth.

	T1	**T2**	**T3**	**T4**
Dry basis composition (%)				
Ground corn	63.0	61.0	65.3	64.7
Soybean paste	11.0	14.0	5.7	6.3
Crotalaria 30 d	20.0	-	-	-
Crotalaria 40 d	-	20.0	-	-
Chipile 30 d	-	-	20.0	-
Chipile 40 d	-	-	-	20.0
Star grass	3.0	2.0	6.0	6.0
Urea	1.0	1.0	1.0	1.0
Mineral mix*	2.0	2.0	2.0	2.0
Chemical composition (calculated)				
ME (Mcal kg^{-1} DM)	2.81	2.81	2.83	2.83
Crude protein (%)	17.0	17.0	17.0	17.0
Neutral detergent fiber (%)	23.6	23.8	21.2	21.3

*17.58% calcium, 2.40% phosphorus, 36.50% sodium chloride, 11.70% sulfur, 0.71% zinc, 0.14% copper, 0.0007% iodine, 0.0016% cobalt, 0.0029% selenium, 0.024% fluorine, and 5.0% moisture.

USA) for 15 min at 121 °C and 15 psi], 5 mL of mineral solution I [6 g K_2HPO_4 (J. T. Baker®) in 1,000 mL of distilled water], 5 mL of mineral solution II [6 g KH_2PO_4 (J. T. Baker®)+$(NH_4)_2SO_4$ (J. T. Baker®)+12 g NaCl (Meyer®)+2.45 g $MgSO_4$ (Meyer®)+1.6 g CaCl-$2H_2O$ (Meyer®) in 1,000 mL of distilled water], 0.1 mL of 0.1% resazurin (Sigma-Aldrich®), 0.2 g of soy peptone (MCDLab®), 0.1 g yeast extract (BD Bioxon®), 4 mL cysteine-sulfide solution [3.125 g L-cysteine (Sigma-Aldrich®) in 15 mL 2N NaOH (Meyer®)+3.125 g Na_2S-$9H_2O$ (Meyer®) with a graduate mark of 250 mL of distilled water], 5 mL of 8% Na_2CO_3 solution (J. T. Baker®), and 50.6 mL of distilled water. The medium was sterilized for 15 min in an autoclave at 121 °C and 15 psi, according to the modifications proposed by Sánchez-Santillan *et al.* (2016) to the methodology of Cobos and Yokoyama (1995).

A 120-mL glass sample vial with 0.5 g of DM from a treatment and 45 mL of culture medium was considered a biodigester and experimental unit. The vials were kept under anaerobic conditions with CO_2, hermetically sealed with a 20-mm Ø neoprene stopper and an aluminum ring. The biodigesters were sterilized for 15 min at 121 °C and 15 psi. The biodigesters were inoculated with 5 mL of total ruminal bacteria obtained from the rumen fluid of a Suiz-bu cow and incubated at 39 °C for 72 h in a hot water bath. The cow grazed in pangola grass grasslands before the sample of the rumen fluid was taken. The rumen fluid was centrifuged at 1,157 *x g* for 3 min to precipitate protozoa and fiber particles (Torres-Salado *et al.*, 2019).

Biogas production was measured by displacing the plunger of a 50-mL glass syringe (BD Yale®, Brazil). Biogas was measured at 2, 4, 6, 8, 10, 12, 24, 48, and 72 h and the accumulated production was reported. The values of the accumulated biogas production were used to estimate the biogas production kinetics, using the Gompertz model:

$$Y = A * \left\{ \exp\left[-b * \exp\left(-k * t \right) \right] \right\}$$

Where: Y=volume of biogas at time t (mL g^{-1} of DM), A=total biogas production potential when $t=\infty$ (mL g^{-1} of DM), b=constant rate of biogas production of the potentially degradable material (mL h^{-1}), k=lag time (h), microbial efficiency constant factor, defined as the intercept of the time axis of the tangent line at the inflection point, t=incubation time (Lavrenčič *et al.*, 1997).

At 72 h of incubation, the pH was measured with a potentiometer (Hanna® HI2211, Italy; calibration: pH 7 and 4). The residual sample of the biodigester was filtered in ANKOM® F57 bags with constant weight. The bags with the residue were dried for 24 h at 60 °C in a drying oven. ANKOM® bags were heat sealed and NDF and ADF content was determined (Van Soest *et al.*, 1991). The degradation of dry matter (DMD), of NDF (NDFD), and of ADF (ADFD) were estimated by difference (Hernández-Morales *et al.*, 2018). The models described by Menke *et al.* (1979) were used to estimate organic matter digestibility (OMD) and metabolizable energy (ME). Additionally, the model developed by Getachew *et al.* (2004) was used for short-chain fatty acids (SCFA).

Statistical analysis

The experimental design was completely randomized with 10 replications per treatment. Data on biogas production, biogas production kinetics, and *in vitro* fermentative characteristics were analyzed with the GLM procedure (SAS Institute Inc., 2011). The mean values were compared with Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The chipile treatment with 40 d of regrowth (T4) recorded the highest production of accumulated biogas, 2 to 24 h after incubation ($p \leq 0.05$). Meanwhile, 40-d crotalaria (T2) and 30-d chipile (T3) did not show differences 2, 4, 6, 8, and 10 h after incubation (Table 3). Therefore, T4 contained a greater amount of non-structural carbohydrates available for fermentation, since this type of carbohydrate (Texta *et al.*, 2019) and the protein fraction (Rodríguez *et al.*, 2010) are fermented in the first 24 h.

At 48 h, chipile treatments (T3 and T4) recorded an average of 152.35 mL g^{-1} DM: 12.3% more biogas than the average of crotalaria treatments. However, at 72 h, the 30-d crotalaria treatment (T1) produced the highest volume of accumulated biogas. This represented 12.3% more biogas than the average of the chipile treatments (T3 and T4). It can be deduced that structural carbohydrates of crotalaria with 30 d of regrowth are more susceptible to fermentation by cellulolytic bacteria than those contained in chipile, since such carbohydrates are fermented after 24 h (Texta *et al.*, 2019). Espinoza-Sánchez *et al.* (2020) reported higher *in vitro* biogas production values (242.3 mL g^{-1} DM) than those recorded in this study for sheep diets containing 40% corn grain, 1% urea, 25% *Samanea saman* pod, 6% molasses, 26% pangola grass, and 2% mineral mix. Those values can be attributed to the availability and nature of the carbohydrates included in the diets evaluated by both authors, since biogas production is the result of their fermentation (Amanzougarene and Fondevila, 2020).

The estimators of the fermentation kinetics show that the treatment that contained crotalaria with 30 d of regrowth (T1) produced the highest value of A ($p \leq 0.05$) (Table 4).

Table 3. Biogas production (mL g^{-1} DM) of diets that include 20% crotalaria or chipile with 30 or 40 d of regrowth.

Incubation time (h)	T1	T2	T3	T4	SEM
2	7.5 c	16.1 b	15.3 b	20.9 a	0.86
4	20.5 c	28.4 b	28.8 b	40.6 a	1.25
6	33.2 c	42.0 b	41.6 b	59.2 a	1.61
8	50.7 c	56.5 b	58.9 b	79.7 a	1.86
10	67.3 b	65.9 b	70.5 b	90.9 a	1.74
12	80.3 b	72.5 c	81.3 b	98.9 a	1.69
24	115.1 b	99.7 c	117.9 b	124.5 a	1.61
48	141.0 b	130.4 c	150.0 a	154.7 a	1.66
72	185.3 a	145.6 c	163.5 b	166.5 b	2.39

[a,b,c] Means with different letters within a row are different ($p \leq 0.05$). SEM=standard error of the mean. T1=crotalaria with 30 d of regrowth; T2=crotalaria with 40 d of regrowth; T3=chipile with 30 days of regrowth; T4=chipile with 40 days of regrowth.

This result indicates greater biogas production, which matches the direct measurement of biogas (Table 3). Therefore, the treatment containing crotalaria with 30 d of regrowth (T1) showed the greatest availability of carbohydrates for in vitro fermentation (Amanzougarene and Fondevila, 2020). However, estimator b showed that the 40-d chipile (T4) treatment had a better biogas production rate, although it did not produce the greatest volume of the said gas. This indicates that its passage through the rumen would be faster than the rest of the treatments, as a result of its fermentation rate (b) (Table 4) and biogas production at 24 h (Table 3).

The *k* estimator refers to the time it takes for the microorganisms to adhere to the substrate; consequently, 40-d crotalaria (T2) had the lowest *k* (Table 4). Overall, none of the treatments in this study had the best kinetic estimators. The qualities of each treatment depend on the ingredients it contains. Crotalaria and chipile, as well as their age at regrowth, influence these estimators.

Crosby *et al.* (2017) reported higher estimator values (A, *b*, and *k*) than the values recorded in this study (Table 4) for diets containing 40% corn stover, 30% winter squash dry residue, 18% ground corn, 10% soybean paste, 1% urea, and 1% mineral mix. For their part, Rodríguez *et al.* (2017) recorded similar values to those of the present study: similar for A, higher for *k*, and lower for *b*; their study diet contained 67.5% grain sorghum, 5% alfalfa hay, 11% soybean paste, 4% molasses, 10% corn stover, 1.5% urea, and 1% mineral mix. The difference between the values of the estimators reported in this study and the values recorded by the abovementioned authors may be the result of two factors: a) nutritional composition of the treatments, and b) methodology used to establish the *in vitro* gas production.

Table 4. Fermentation kinetics and in vitro fermentative characteristics of diets that include 20% crotalaria or chipile with 30 or 40 d of regrowth.

Variables	T1	T2	T3	T4	SEM
A (mL g^{-1} DM)	163.9 a	135.9 c	154.8 b	153.7 b	1.77
b (mL h^{-1})	0.101 c	0.107 bc	0.112 b	0.151 a	0.031
k (h)	2.83 a	2.37 d	2.63 b	2.50 c	0.003
pH	6.9 a	6.7 b	6.8 a	6.8 ab	0.01
DMD (%)	69.0 a	50.4 b	67.9 a	67.2 a	1.86
NDFD (%)	56.7 a	35.7 c	58.7 a	48.6 b	2.28
ADFD (%)	65.9 a	10.2 c	49.6 b	43.4 b	4.82
ME (Mcal kg^{-1} DM)	1.49 b	1.31 c	1.66 a	1.65 a	0.03
OMD (%)	43.0 b	37.6 c	48.3 a	48.0 a	1.07
SCFA (mmol g^{-1} DM)	2.50 a	2.15 b	2.65 a	2.65 a	0.05

[a,b,c] Means with different letters within a row are different ($p \leq 0.05$). SEM=standard error of the mean. T1=crotalaria with 30 d of regrowth; T2=crotalaria with 40 d of regrowth; T3=chipile with 30 days of regrowth; T4=chipile with 40 days of regrowth; A=total biogas production potential; *k*=lag time; *b*=constant rate of biogas production of the potentially degradable material; pH=potential of hydrogen; DMD=dry matter degradation; NDFD=Neutral Detergent Fiber Degradation; ADFD=acid detergent fiber degradation; ME=metabolizable energy; OMD=organic matter degradation; SCFA=short chain fatty acids.

After 72 h of incubation, the pH value of the culture medium of the treatments ranged from 6.7 to 6.9 (Table 4). Those values are within the range required for the growth of ruminal microorganisms (Nagaraja, 2016). Therefore, the crotalaria or chipile fermentation products did not take the pH values beyond the standards for ruminal bacteria. *In vitro* degradation determinations are useful because they establish a correlation with *in vivo* digestibility (Gosselink *et al.*, 2004). The treatment that contained crotalaria with 40 d of regrowth (T2) showed the lowest dry matter degradation (DMD) and organic matter degradation (OMD) ($p \leq 0.05$), while the rest of the treatments did not record DMD differences ($p > 0.05$) (Table 4). Meanwhile, chipile treatments (T3 and T4) recorded higher OMD ($p \leq 0.05$) (Table 4).

The 40-d crotalaria treatment (T2) had the least degradation of detergent fibers (NDFD and ADFD) (Table 4). In addition, treatments with 30 d of regrowth (T1 and T3) showed the highest NDFD ($p \leq 0.05$), with no difference between them ($p > 0.05$). Likewise, the crotalaria treatment with 30 d of regrowth (T1) showed the highest ADFD ($p \leq 0.05$). The changes in fiber degradation are assumed to be caused by the state of maturity of the regrowth, since it recorded greater physiological changes at 40 d, due to the lignification process that hindered the adherence of ruminal bacteria (Hoffman *et al.*, 2007). Espinoza-Sanchez *et al.* (2020) reported higher DMD values (74.9%) than all the treatments of the present study and lower NDFD values (54.4%) than the 30-d chipile (T3) and crotalaria (T1) treatments for sheep diets that include 50 % ground ripe mango, 1% urea, 25% *Samanea saman* pod, 6% sugar cane molasses, 16% pangola grass, and 2% mineral mix.

Chipile treatments (T3 and T4) showed higher ($p \leq 0.05$) content of metabolizable energy (ME) and short-chain fatty acids (SCFA) (Table 4). This indicates that chipile treatments showed a higher energy contribution to the microbial fermentation and a better production of SCFA, as fermentation products under the *in vitro* conditions of the present experiment. SCFA and biogas production showed the same trend, since biogas production is the result of the fermentation of carbohydrates and their transformation into volatile fatty acids (Amanzougarene and Fondevila, 2020). Espinoza-Sánchez *et al.* (2020) recorded higher ME values than those reported in this study for sheep diets using ripe mango as an energy source and *Samanea saman* pod as a protein source.

CONCLUSIONS

Given their biogas production and *in vitro* fermentative characteristics, sheep diets containing 20% chipile or crotalaria with 30 d of regrowth proved to be an alternative in the elaboration of whole food diets for the intensive fattening of lambs in the tropics. However, further studies are required to prove the feasibility of producing these legumes as sheep feed.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Genetic resources and product diversification in a transitioning coffee agroecosystem in Mecayapan, Veracruz, Mexico

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ABSTRACT

Objective: To analyze a coffee agroecosystem undergoing an agroecological transition —considered as an alternative for the efficient use and the conservation of resources— through an agroecological association design with three legumes and one cucurbit in coffee cultivation.

Design/Methodology/Approach: Collection and identification of genetic resources found in the agroecosystem; agroecological association design with three legumes and one cucurbit in a coffee crop; zig-zag soil sampling for physical, chemical, and biological analyses; data analysis using JASP software version 0.16.2.

Results: We identified 42 weed species with various uses within the coffee agroecosystem, as well as the criteria for the association of species according to use. The soil analysis showed a significant correlation between micronutrients and nitrogen-fixing bacteria. The boron variable also influences the growth of such bacteria.

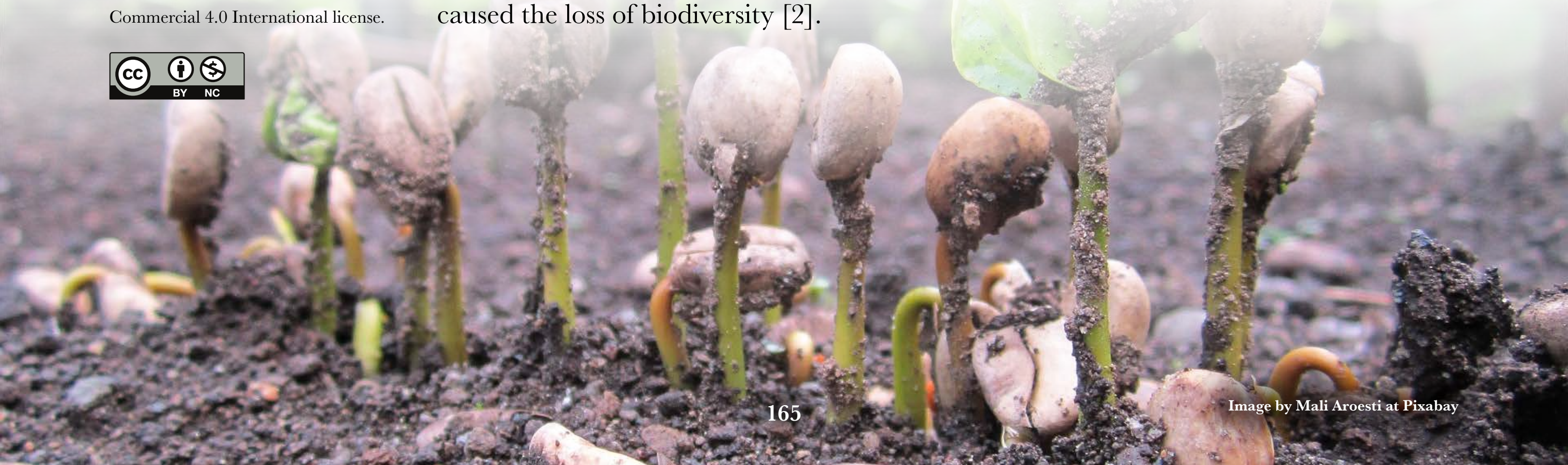
Study Limitations/Implications: The results apply to the agroecological model in transition presented.

Findings/Conclusions: Agroecological transition in coffee agroecosystems is slow but contributes to improving soil conditions. It also allows for the reappearance of usable weed species. There is a significant correlation between boron and the development of nitrogen-fixing bacteria. Likewise, the physical properties of soil have a direct impact on the growth of such bacteria.

Keywords: agroecology, transition, agroecosystem.

INTRODUCTION

Over the last decades, coffee agroecosystems have endured a bleak outlook as a consequence of the crisis caused by the drop in the price of grain in international markets. This situation led to the abandonment of farms and the appearance of diseases such as rust [1]. It also resulted in a change in land use to grow new crops, a process that, in its turn, caused the loss of biodiversity [2].



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Coffee-growing is a major activity in several indigenous regions of Mexico. Coffee has great economic, environmental, and cultural relevance in shaded agroecosystems. However, despite its wide biocultural and genetic diversity, the use of crop association is currently limited in the coffee-growing agroecosystems of Mecayapan, Veracruz. In this context, we must also take into account the drop in prices and the appearance of diseases, as well as the high production costs and the promotion of monoculture.

Agroecosystems can be defined as ecosystems "modified by human beings to a certain degree in order to use natural resources in production processes," satisfying food, fibers, medicine, and timber needs [3]. Agroecosystems aim to conduct agricultural activity in such a way that an efficient and sustainable production is achieved, adequately and fairly exploiting resources without exceeding their load capacity [4].

Agroecosystem design is based on local knowledge about the use, function, importance, and characteristics of species. This subject has been barely studied from an ethnobiological approach. Ethnobiology is based on peasants knowledge about the use, interactions, and reciprocal relations between nature and human beings. The dependence on technological packages, the pollution of aquifers, erosion, eutrophication, and loss of native germplasm of cultivated species have modified the operation and structure of agroecosystems [5]. For example, the loss of microorganisms affects ecosystems, because several processes depend on them, such as the cycle of C, N, and other nutrients, animal and plant health, and agriculture.

Agroecology seeks to "restore flows of energy and nutrients within ecosystems and agroecosystems to maintain a dynamic balance between their constituting elements" [6]. This approach goes beyond organic production. It is a transdisciplinary scientific effort that strives to bring together traditional and scientific knowledge to solve problems affecting specific environments. Therefore, it must consider any phenomenon relating to the management and use of natural resources [7]. According to Zambrano [8], as an interdisciplinary science, agroecology requires participatory processes that involve all parties; likewise, it needs to develop proposals that impact the social, economic, and productive spheres.

Therefore, agroecology proposes an analysis that allows us to conceive agroecosystem design as a response to rural problems, focusing on the production of healthy food through the implementation of peasant knowledge and organization structures [9]. Agroecology also entails reclaiming a vast lore guarded by communities about the use and management of local species [10].

Safeguarding biodiversity is essential to maintain the dynamic balance of agroecosystems. Their conservation requires the design of strategies that enable the study, protection, and adequate use of species, without exceeding their load capacity —for example, creating seed repositories. One of the multiple benefits of conservation is the upkeeping of whole sets of species, populations, communities, and ecological services within the agroecosystem [11]. Consequently, proposals that respond to productive, economic, and social needs should be developed. These proposals should be based on agricultural lore and its application to the diversification of coffee agroecosystems with local species.

MATERIALS AND METHODS

This work was conducted in a 2.5-ha plot transitioning towards agroecology, located in Mecayapan, Veracruz, Mexico. This region is inhabited by indigenous Nahuas and Popolucas, who typically practice agricultural activities in maize fields, coffee-growing, and, on a smaller scale, husbandry. Mecayapan is located in the Santa Marta Sierra, at 18° 13' 10.71" N and 94° 50' 14.36" W. It is adjacent to the municipality of Tatahuicapan de Juárez to the east and the municipality of Soteapan to the west. It has an altitude of 360 m.a.s.l. [12].

To conduct our research, we used two methods to identify the number of genetic resources that can be found in coffee agroecosystems undergoing ecological transition. We put in place a diversified design and carried out soil analyses to determine the correlations between physical, chemical, and biological parameters present in associations.

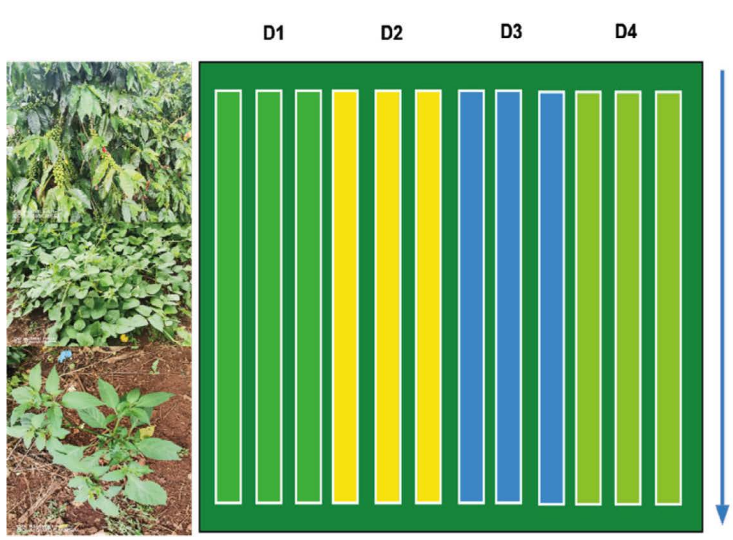
Collection of weeds. We collected plants to obtain a record of species diversity. We collected plants with flowers and subsequently spread them out on paper, in order to maintain their quality. Once each species was placed on a sheet of paper, we pressed and stored them [13]. Afterwards, the specimens were identified and grouped according to their environmental, social, and cultural use [14].

Agroecological design. We developed an agroecological design based on the interaction of three legumes (*Vigna unguiculata*, *Pachyrhizus erosus*, *Arachis hypogaea*), one cucurbit (*Cucurbita argyrosperma*), and native black and guajolotero maize (*Zea mays*) (Figure 1). Soil sampling surveys were conducted in 30 m×10.20 m (length×width) growing blocks.

Count of free-living nitrogen-fixing bacteria (BFNVL). We used the viable count method by serial dilution ($1/10^4$), with 10 g of soil in a combined carbon culture medium [15].

Soil analysis. Composite samples were obtained for each agroecological design using a zig-zag sampling method. Initial and final soil samples were collected (1 kg per design).

Data analysis. The following parameters were taken into consideration: field capacity, permanent wilting point (PMP), hydraulic conductivity, apparent density, pH,



Design	Species in association
1	Coffee, corn and peanuts
2	Coffee, corn and beans
3	Coffee, corn and pumpkin
4	Coffee, corn and jícama

Figure 1. Coffee agroecosystem design with native species.

total carbonates, organic matter (OM), P-Bray, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, B, and N-NO_{3-}. They were analyzed before and at the end of cultivation, estimating the percentage variation of each property by association. Statistical data were analyzed with JASP software version 0.16.2, using a Pearson linear correlation.

RESULTS AND DISCUSSION

Agroecological transition models have been promoted as an alternative to face high production costs. Model diversification has also been presented as a biodiversity conservation strategy. Their goal is to seek efficient exploitation and use of local resources by changing some production paradigms and giving priority to the potential relations between individuals within the agroecosystem.

Genetic resources in the transitioning agroecosystem

A total of 42 plant species, grouped into 25 families, were identified at the Mecayapan coffee plantation undergoing an agroecological transition. Fabaceae, Asteraceae, Euphorbiaceae, and Poaceae are some of the most abundant families in the region (Table 1).

The use value of all species in the study area were classified as follows: twelve can be used as green matter (incorporating them into the soil); nine species have various uses; eight are edible; six are used as cover crops to protect the soil and control other weeds; and four have medicinal use, mainly as antiseptics, diuretics, and treatment for intestinal and urinary infections (Table 2).

Use level is distributed into four main categories:

Environmental: Species used as cover crops for soil protection and species pruned and mixed as organic matter into the soil.

Environmental-economic: Species used for shade that also have other uses (*e.g.*, firewood, fodder, medicine, and food).

Social: Edible species.

Cultural: Species used for medicinal, artisanal, and culinary purposes.

Identified species are sorted into three main groups according to their usable parts: leaves, the whole plant, and fruits (Table 3).

In agroecological transition models, a natural process of biotic regulation takes place, contributing to the appearance of various species that had been disturbed by the use of synthetic products or by some other activity [16]. Associated crops within these models allow for more interaction between species. The number of weeds in the agroecosystem plays an important environmental role, since weeds tend to grow in disturbed soils and are considered indicators of soil health [17].

Traditional association in coffee agroecosystems responds to the need to improve production and allows producers to obtain other valuable products. Coffee systems may include timber, fruit-bearing, edible, medicinal, ornamental, and construction species. There are also double-purpose species that provide a product and fix atmospheric nitrogen, thus contributing to the improvement of soil [18]. A relevant criterion for

Table 1. Frequencies per amount of species/family.

Species/families	Frequency	Percentage	Valid percentage	Accumulated percentage
Asteraceae	3	7.143	7.143	7.143
Boraginaceae	1	2.381	2.381	9.524
Amaranthaceae	1	2.381	2.381	11.905
Brassicaceae	1	2.381	2.381	14.286
Chochlospermaceae	1	2.381	2.381	16.667
Convolvulaceae	1	2.381	2.381	19.048
Cucurbitaceae	1	2.381	2.381	21.429
Cyperaceae	1	2.381	2.381	23.810
Dennstaedtiaceae	1	2.381	2.381	26.190
Euphorbiaceae	3	7.143	7.143	33.333
Fabaceae	8	19.048	19.048	52.381
Fagaceae	1	2.381	2.381	54.762
Iridaceae	1	2.381	2.381	57.143
Leguminosae	1	2.381	2.381	59.524
Lygodiaceae	1	2.381	2.381	61.905
Malpighiaceae	1	2.381	2.381	64.286
Malvaceae	2	4.762	4.762	69.048
Marantaceae	1	2.381	2.381	71.429
Oxalidaceae	2	4.762	4.762	76.190
Passifloraceae	1	2.381	2.381	78.571
Plantaginaceae	1	2.381	2.381	80.952
Poaceae	3	7.143	7.143	88.095
Rubiaceae	2	4.762	4.762	92.857
Solanaceae	2	4.762	4.762	97.619
Vitaceae	1	2.381	2.381	100.000
Total	42	100.000		

Table 2. Use frequency of genetic resources found in the agroecosystem.

Use	Frequency	Percentage	Valid percentage	Accumulated percentage
Edible	8	19.048	19.048	19.048
Medicinal	4	9.524	9.524	28.571
Handicraft	3	7.143	7.143	35.714
Cover	6	14.286	14.286	50.000
Firewood, forage, medicinal, edible	9	21.429	21.429	71.429
Organic matter	12	28.571	28.571	100.000
Total	42	100.000		

Table 3. Usable parts frequency.

Usable part	Frequency	Percentage	Valid percentage	Accumulated percentage
Leaves	15	35.714	35.714	35.714
Fruit	6	14.286	14.286	50.000
Root	1	2.381	2.381	52.381
Bole height	3	7.143	7.143	59.524
Leaves and seeds	2	4.762	4.762	64.286
Whole plant	15	35.714	35.714	100.000
Total	42	100.000		

crop association is avoiding direct competition for light and nutrients among the species. When they first establish the associations, coffee producers at Mecayapan take into account this criterion.

Analysis of physical, chemical, and biological parameters in soils with crop association

Twenty-one variables were studied using the JASP software. The soil analyses provided results for five physical parameters and 16 chemical parameters that enabled the identification of important variables for the further development of BFNVL. There is a strong correlation between the saturation point (Figure 2a) and PWP regarding the growth of BFNVL (Figure 2b). Regarding peanut, corn, and coffee association, the following decrease percentages were recorded: saturation (5.71%), field capacity (5.59%), and PWP (5.38%). These decreases could affect the biological activity of the soil.

The P-Bray results show a significant positive correlation with BFNVL —*i.e.*, the higher the amount of P-Bray, the greater the development of BFNVL (Figure 2c). In the peanut, corn, and coffee association, a 558% decrease was observed. Growing peanuts requires high P and K contents. This crop exhausts the said elements, resulting in a decrease [19].

B has a strong correlation with the development of biological activity, in this case, BFNVL (Figure 2d). In this study, the rest of the parameters did not have a significant correlation ($p \geq 0.05$) with the development of BFNVL.

Most macronutrients had a significant positive correlation (Figure 3a) —*i.e.*, the greater the number of macronutrients, the greater the biological activity [20]. However, the peanut, corn, and coffee association in this analysis recorded the highest decrease in macronutrients (234.9%), as a consequence of the nutritional requirements of the crop [21].

Micronutrients showed a moderate, but not significant, correlation with BFNVL (Figure 3b). These variables play a key role in the increase of the number of bacteria in the soil. The various associations in which the initial and final amounts of micronutrients showed a decrease include squash, corn, and coffee (71.2%) and peanut, corn, and coffee (51.8%). The highest percentage of macronutrient extraction occurred in the peanut, corn, and jicama association (234.9%).

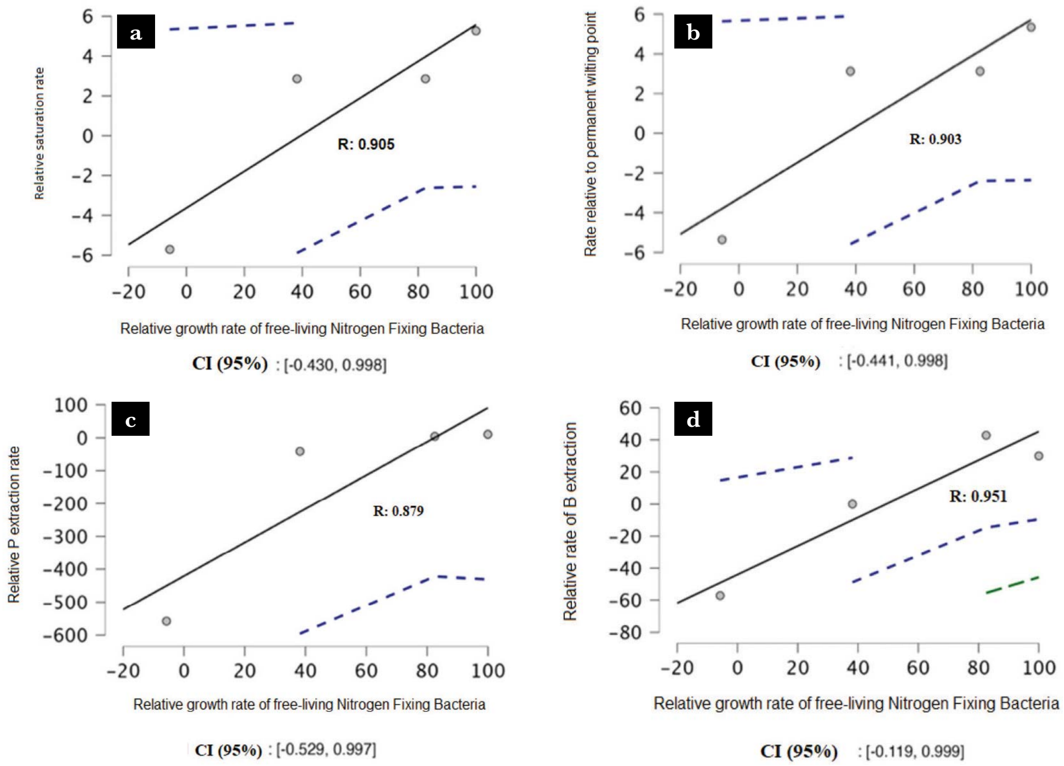


Figure 2. Diagram of the correlation between free-living nitrogen-fixing bacteria (BFNVL) and the degree of variation of the saturation point (a) and permanent wilting point (PWP) (b), Bray phosphorus (P-Bray) (c), and boron (d), at the beginning and end of cultivation.

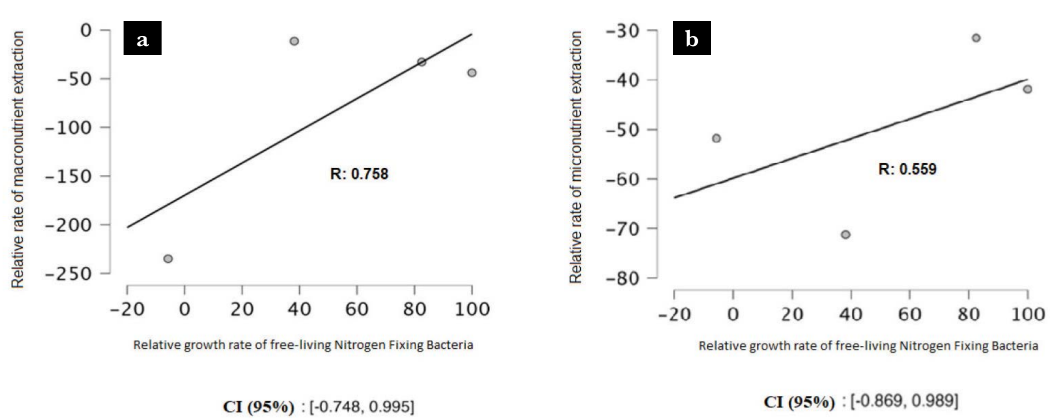


Figure 3. Diagram of the correlation between free-living nitrogen-fixing bacteria (BFNVL) and the degree of variation of macronutrients (a) and micronutrients (b), at the beginning and end of cultivation.

The association that caused the greatest modification to physical properties was jicama, corn, and coffee (37.3 %). Meanwhile, the same association extracted the highest amount of pH, carbonates, and CE (1,614.7 %).

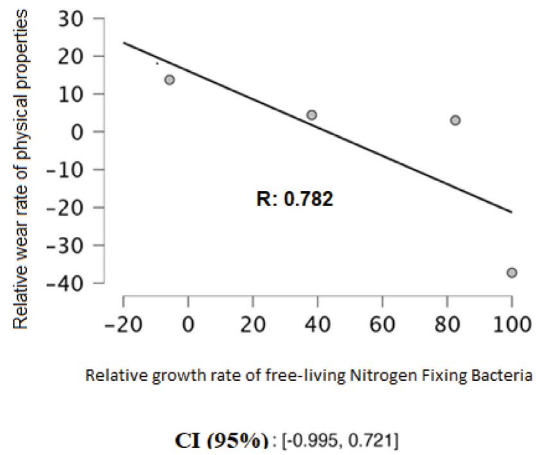


Figure 4. Diagram of the correlation between free-living nitrogen-fixing bacteria (BFNVL) and the degree of decrease of physical properties, at the beginning and end of cultivation.

CONCLUSIONS

The use of agrochemicals in this coffee agroecosystem was suspended three years ago. This situation has contributed to the reappearance of edible, medicinal, and artisanal use species, thereby increasing the diversity of local flora and fauna.

To establish a diversified association model for agroecological transition, the nutritional requirements of the species involved must be taken into consideration, since many of them extract macro and micronutrients from the soil, which in turn leads to degradation by absorption.

The beans, corn, and coffee association absorbed the lowest percentage of micro and macronutrients.

B is a micronutrient that has a strong correlation with BFNVL and directly impacts their development.

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