

AGRO PRODUCTIVIDAD

Evaluation of the
Genipa americana L. /
Heliconia stricta Huber
 agroforestry system and its
 effects on soil fertility

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Agradecimientos: Son opcionales y tendrán un máximo de tres renglones para expresar agradecimientos a personas e instituciones que hayan contribuido a la realización del trabajo.

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Diversity and relative abundance of rodent species under three habitat conditions in the Altiplano Potosino Oeste, Mexico

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ABSTRACT

Objective: To evaluate the diversity and abundance of rodent species, as well as vegetation cover, under three habitat conditions in the Altiplano Potosino Oeste.

Design/Methodology/Approach: The work was carried out in eight sites with good (3), regular (2), and poor (3) vegetation cover conditions. Frequency, basal, and aerial cover data were collected in three plot sizes to obtain the results for the three types of vegetation: arborescent stratum (20×20 m), shrubs (3 plots of 5×5 m), and herbaceous stratum (2 plots of 1×1m). In order to collect the rodent data, three sampling nets with 100 Sherman traps were used. Rodent species diversity was determined using the Shannon-Whinner index (SWI), while abundance was obtained using the Relative Density Index (RDI). Differences were determined using ANOVA.

Results: Grass cover decreased depending on the condition; however, this was not the case of the shrub cover. The overall diversity of rodent species was 21 (16 in fair, 15 in good, and 14 in poor condition). The total SWI and RDI decreased depending on the condition. *Dipodomys ornatus*, *D. merriami*, and *Chaetodipus nelsoni* were the most abundant and most widespread species.

Study Limitations/Implications: The study was conducted during the most restrictive season and under drought conditions. This situation impacted cattle, but not rodents. However, although their plasticity maintained relatively stable RDI values, the indices decreased depending on the condition.

Findings/Conclusions: Despite their habitat conditions, the rodents found in the area are more diverse and abundant than those reported in reference works. Therefore, further studies about this subject should be carried out using rodents as bioindicators.

Keywords: *Dipodomys ornatus*, *D. merriami*, *Chaetodipus nelsoni*, Relative Density Index, Shannon-Wiener Index.

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INTRODUCTION

Livestock practices in arid and semi-arid areas are usually inadequate and lead to vegetation cover degradation, which increases under adverse weather conditions (Holechek and Valdez, 2018; Weber-Grullon *et al.*, 2022). Such is the case of the Altiplano Potosino in Mexico, where 73% of the



surface is used for an extensive cow-calf production system (INEGI, 2022). In this area, the livestock density and the erosion rate are high; additionally, there is a forage deficiency. These conditions lead to an increase in shrub species with low forage value (Li *et al.*, 2022), low productivity, a poor nutrient value, and a poor body condition of the livestock (Chen *et al.*, 2022). In addition to livestock practices, the herbivory of wild rodents can impact the structure and regeneration of vegetation (Lucero and Callaway, 2018; Zhong *et al.*, 2022), which can be worsened by weather conditions (Peterson *et al.*, 2021).

In arid areas of North America, 50% of the wild rodent species are granivorous (Fox, 2011). Rodents have an important ecological role as seed dispersers, they establish vegetation patches (Godó *et al.*, 2022), turn over the soil (Eldridge *et al.*, 2012), and are a key link in the food chain (Fox, 2011). In semi-arid and arid ecosystems, the specific composition of rodent communities includes a great number of individuals of one or several predominant species, while other species are hardly included in this type of communities (Hernández *et al.*, 2011; Lightfoot *et al.*, 2012). This structure can be maintained in a relatively regular state through time; however, its abundance and activity areas fluctuate depending on precipitation and, therefore, on vegetation biomass and cover (Lightfoot *et al.*, 2012; Bai *et al.*, 2022).

In addition to overgrazing and its consequences on natural resources, drought impacted the Altiplano Potosino Oeste (APO) in 2010 and 2011, causing weight loss and mortality among livestock (Servicio Meteorológico Nacional (México), n.d.). Overall, body condition and survival of livestock are easily noticed, unlike other users of the ecosystem (*e.g.*, rodents). Therefore, the objective of this study was to evaluate the diversity and the relative abundance of rodent species, under three vegetation cover conditions of the APO during droughts.

MATERIALS AND METHODS

The APO includes the municipalities of Salinas, Villa de Ramos, and Santo Domingo, in the NW portion of San Luis Potosí. APO is located between the following coordinates: 22° 46' - 23° 80' N and 101° 0' - 102° 25' W. The climate is dry semi-warm with summer rains (BS₀kw); the mean annual temperature ranges from 12 to 18 °C, while the mean annual precipitation fluctuates between 300 to >400 mm. The 2010 mean annual precipitation reached 270 mm; however, in 2011 only 83 mm were recorded (SMN, 2022). The microphyllous desert scrub prevails in the area, with various degrees of grass cover (INEGI, 2022). Eight livestock sites from the APO area were selected to conduct the sampling field work. They were at least 10 km apart from each other. The sites were classified as follows: three had a good vegetation cover (G), two had a regular vegetation cover (R), and three had a poor vegetation cover (P). Additionally, each site was qualified according to the body condition of the livestock, based on the following scale: 1 (skinny); 2 (thin); 3 (moderate); 4 (good); and 5 (fleshy) (Houghton *et al.*, 1990).

Field work was carried out from March to May 2011, when rodents faced the most restrictive availability of resources (Lightfoot *et al.*, 2012). Vegetation cover was evaluated using 20×20 m plots nested at random, with two replicates per site. Frequency, basal cover, and area of the arborescent species were determined. The same variables were used

to measure shrub species in three 5×5 m plots, located within the abovementioned plots. Perennial grasses and herbs were established in two 1.0 m² plots (Stohlgren *et al.*, 1999). Cover differences were calculated with an ANOVA, while a Tukey's Test (Statistical Analysis System [SAS], 2004) was used for the comparison of means. Arborescent stratum was recorded only in P and it was not used in the comparisons. Rodents' population sampling was carried out using three trapping nets per site. Each net contained 100 Sherman traps (10×10 lines, placing a trap every 10 m) (Krebs, 1989), which remained active for 72 h. The bait consisted of oat flakes and the traps were checked at dawn and dusk. The sampling effort was carried out during 7,200 days trap⁻¹. The identification keys developed by Mellink (1984) and Álvarez-Castañeda *et al.* (2017) were used to determine the rodent species. The Shannon-Whinner (H') index was used to determine the abundance of species per condition, using the Ecological Methodology 6.0 software (Kenney and Krebs, 2001). The abundance of the rodent population was evaluated using the Relative Density Index (RDI), following the methodology proposed by Calhoun (1959). This methodology compares the number of captures with the number of night traps and the sampling area. The result is set forth as individuals per hectare (Ind ha⁻¹).

RESULTS AND DISCUSSION

The basal and aerial covers of grasses and herbs were higher (Table 1) with the highest values of G (P=0.0007), where livestock had a regular body condition. In R and P, a lower vegetation cover, livestock with thin body condition, and livestock mortality were observed. According to White (2008), these results can be the consequence of drought and overgrazing. The basal cover in the shrub stratum was higher in inverse proportion to habitat quality. There were no differences between the aerial covers (P=0.0005). The arborescent stratum was scarce. None of the conditions recorded an optimum grass cover. G had a regular condition, while in the others, according to Holechek *et al.* (1989) and Ahumada-Hernández *et al.* (2022), the condition was poor.

Table 1. Cover (%) of the vegetation strata (basal and aerial covers), per habitat condition in the Altiplano Potosino Oeste (G=Good, R=Regular, P=Poor).

Category	Layer	Vegetation coverage (%)	
		Basal	Aerial
G	Grasses and herbs	33.36 ^a	55.34 ^a
R		11.67 ^b	22.68 ^b
P		7.62 ^b	18.54 ^b
G	Shrub	0.06 ^c	3.85 ^c
R		0.07 ^c	2.73 ^c
P		0.28 ^c	6.05 ^c
G	Arborescent	<0.01 ^c	<0.10 ^c
R		<0.01 ^c	<0.10 ^c
P		0.04 ^c	0.16 ^c

a,b,c Values in the same column with different letter are different (P>0.05).

In the arid areas, regardless of their combination, approximately 11 rodent species can usually be found in a community (Brown and Kurzius, 1987). For this work, we captured 21 species. This number is higher than the 15 rodent species recorded by Mellink and Valenzuela (1995) for the Altiplano Potosino. It is also higher than the results of Hernández *et al.* (2011) for the Mapimí region (Durango, México), the 14 rodent species found by Lightfoot *et al.* (2012) in La Jornada (New Mexico), and the 10 species registered by Ahumada-Hernández *et al.* (2022) in southwestern Zacatecas. However, as a result of the habitat condition, the abundance of rodents was similar to the number recorded by the abovementioned authors: 16 species in R, 15 in G, and 14 in P (Table 2). Based on the Shannon-Wiener index, the diversity of the species depended on the habitat condition (H': G=3.69; R=3.31; P=2.53), because this index weights up the number of species and their abundance (Krebs, 1989).

Regarding the RDI (Table 2), there was no significant differences between basal cover (P=0.606) and aerial cover (P=0.293); however, there was a weak relation between the habitat condition and the population density ($R^2=4.99$). For their part, Ahumada-Hernández *et al.* (2022) reported lower values in the Tula ranch and mentioned a relation between the abundance of rodents and the grass cover; however, no positive correlation was recorded. The following RDI values were obtained: 50.7, 40.9, and 26.7 ind ha⁻¹, for G, P, and R, respectively. Shrub was the predominant type of vegetation in all the conditions; shrubs protect rodents against predators (Lightfoot *et al.*, 2012; Szymański *et al.*, 2020).

Regarding the three habitat conditions, population density was higher than the 17.8 ind ha⁻¹ recorded by Hernández *et al.* (2005) in the Mapimí shrub and the 11.9 ind ha⁻¹ obtained by Lightfoot *et al.* (2012) in La Jornada Basin. Concerning the RDI per species, *D. ornatus* recorded the highest population density in G, followed by *D. ordii*, and *Chaetodipus nelsoni*. In R, the highest population density was obtained by *D. merriami* and *D. phillipsi*, while for P, *D. phillipsi* recorded the highest population density. The species with the lowest RDI in G were: *Peromyscus eremicus* and *Perognathus flavus*. Meanwhile, the lowest population densities in R were registered by: *Ictidomys mexicanus*, *D. nelsoni*, *Peromyscus melanophrys*, *P. maniculatus*, and *P. difficilis*. Finally, the lowest population densities in P were recorded by: *Neotoma leucodon*, *P. eremicus* and *Onychomys torridus*.

Dypodomys merriami and *C. nelsoni* were the most abundant species, in pastures and in shrub, respectively. Meanwhile, *X. spilosoma*, *C. nelsoni*, *D. merriami*, *D. ordii*, *D. ornatus*, *P. maniculatus*, *P. eremicus* and *P. difficilis* were found in the three conditions. However, some of these species had a low population density, particularly *C. nelsoni*, *D. merriami*, *D. ordii* and *D. ornatus*, which were generalist and abundant. These results matched the reports of Hernández *et al.* (2011) and Lightfoot *et al.* (2012), but not the reports of Ahumada-Hernández *et al.* (2022). The species with the lowest presence, found only in one condition and with a low population density, were *L. mexicanus*, *D. nelsoni*, *D. stephensi*, *N. mexicana*, *Peromyscus melanophrys* and *R. megalotis*.

Overall, the species found in the APO seem to be adapted to the conditions of their habitat, where drought is a frequent event. In fact, this work found higher values both for diversity and for population density. The prevailing species were granivorous rodents

Table 2. Rodent species and Relative Density Index (RDI) per habitat condition (Good=G, Regular=R, and Poor=P) in the Altiplano Potosino Oeste.

Family	Species	RDI mean (Ind ha ⁻¹)		
		G	R	P
Sciuridae	<i>Ictidomys mexicanus</i>	-	0.44	-
	<i>Xerospermophilus spilosoma</i>	2.22	2.22	1.78
Heteromyidae	<i>Chaetodipus eremicus</i>	4.00	0.89	-
	<i>C. nelsoni</i>	6.22	1.33	2.67
	<i>Perognathus flavus</i>	0.44	-	0.89
	<i>Dipodomys merriami</i>	4.44	9.78	0.89
	<i>D. nelsoni</i>	-	0.44	-
	<i>D. ordii</i>	7.56	1.78	4.00
	<i>D. ornatus</i>	13.78	2.67	22.22
	<i>D. stephensi</i>	-	1.33	-
	Muridae	<i>Neotoma leucodon</i>	0.89	0.89
<i>N. mexicana</i>		-	1.33	-
<i>Peromyscus melanophrys</i>		-	0.44	-
<i>P. gratus</i>		2.67	-	1.78
<i>P. eremicus</i>		0.44	1.33	0.44
<i>P. maniculatus</i>		1.33	0.44	0.89
<i>P. difficilis</i>		2.22	0.44	2.22
<i>Reithrodontomys fulvescens</i>		-	0.89	1.33
<i>R. megalotis</i>		0.89	-	-
<i>Onychomys arenicola</i>		1.33	-	0.89
<i>O. torridus</i>	2.22	-	0.44	
Total		50.67	26.67	40.89

that store food, mainly *D. ornatus*, *C. nelsoni* and *D. merriami*, as a consequence of the desertification promoted by livestock practices (Graham and Whitford, 2000). Altogether, the composition and the abundance of rodent species in the APO were related to the quality of the habitat and, unlike cattle, rodents do not seem to have been impacted by droughts. Therefore, we recommend considering rodents as bioindicators, carrying out further studies, and extending the sampling collection, both in space and time.

CONCLUSIONS

A higher diversity of rodents and a higher RDI were recorded in this study, both in this region and other regions with similar characteristics. A low body condition and even mortality were recorded among cattle, as a consequence of droughts; however, there was no proof of impact on rodents. Compared with other research, rodent populations were stable; nevertheless, the species diversity depended on their habitat conditions, fluctuating between the highest (good condition) and the lowest values (poor condition). Overgrazing

can pose a risk to the permanence of healthy rodent populations within an ecosystem where the microphyllous shrub prevails.

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Aphid diversity and population fluctuation of vector species of the ringspot virus in papaya (*Carica papaya* L.)

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ABSTRACT

Objective: To examine the diversity of winged aphids in papaya (*Carica papaya* L.) crops and to determine the population fluctuation of vector species of the papaya ringspot virus (PRSV).

Design/Methodology/Approach: We captured winged aphids by placing eight Moericke-type water traps (four green and four yellow). We conducted weekly samplings to locate plants with ringspot symptoms, record the abundance of vector species of PRSV, and document the progress of the disease.

Results: We collected a total of n=694 individuals of 20 species, seven of which fell in the green traps and 19 in the yellow traps. The green traps registered the highest diversity value; however, the yellow traps captured the largest richness. Six of the captured aphids are reported as PRSV transmitters. The analysis of the population fluctuation of the vector species determined that *A. spiraecola* recorded two population peaks during the study: the first at week three, with 93 captured individuals; and the second (and highest) at week ten, with 316 individuals. PRSV was observed in the week seven in nine diseased plants. Full contagion (100%) was reached by week 44.

Study Limitations/Implications: Developing strategies for preventing and controlling pest species and disease vectors crucially depends on the correct choice of methods to capture and monitor insect populations in crops.

Findings/Conclusions: We can infer that vector species conduct short-distance migrations within the plantation. This inference is supported by the increased number of diseased plants.

Keywords: Aphids, crop, disease, insects, virus.

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INTRODUCTION

In Mexico, 20,750 ha are currently used to grow papaya (*Carica papaya* L.), yielding 249,913 tons. The Mexican state of Tabasco holds only 217 ha, in which 3,546 tons are produced. This crop provides a significant income for small producers in the local market. However, papaya plantations are vulnerable to pests and diseases that can considerably reduce their production.



Aphids or plant lice are small-sized insects that measure 2 mm on average. They belong to the order Hemiptera and the infraorder Aphidomorpha (Peña-Martínez *et al.*, 2019). They have a wide geographical distribution, but their diversity is greater in the temperate zone of the northern hemisphere (Trejo-Loyo *et al.*, 2004). Around 4,700 aphid species have been described in the world (Remaudière and Remaudière, 1997; Peña-Martínez *et al.*, 2019). Two-hundred two of those species are distributed in Mexico (Peña-Martínez, 1992b), 53 of which are relevant for agriculture (Peña-Martínez, 1999). Aphids play a major role in the transmission of phytopathogenic viruses (Katis *et al.*, 2007) and some of them are vector species of the papaya ringspot virus. The papaya ringspot virus (PRSV) is considered the main hindrance for the production of this crop (Cabrera-Mederos *et al.*, 2010). It prevents plant growth and considerably reduces the size and quality of the fruit (Yeh *et al.*, 2007), consequently limiting the production of large cultivation areas to only one harvest (Gonsalves, 1998). Therefore, we captured winged aphids with green and yellow Moericke-type traps and analyzed their diversity, in order to determine the population fluctuation of vector species of the ringspot virus.

MATERIALS AND METHODS

The study was conducted from February 1 to November 30 in one hectare of a Maradol papaya cultivation field at Ranchería Macayo, 1st Section, municipality of Huimanguillo, Tabasco, Mexico (INEGI, 2017). The study began when the plants were two months old. A 1.2-m and 2-m distance was established between plants and rows, respectively. Most of the surface of Huimanguillo (95.26%) belongs to the southern Gulf Coastal Plain physiographic region and only 4.74% is found in the Northern Sierras of Chiapas. The municipality has an altitude range from 0 to 1,100 m (INEGI, 2017; Palma-López *et al.*, 2011). It encompasses two climate types: warm-humid with abundant rains in summer (Am) and warm-humid with rain throughout the year (Af). The average annual temperature is 26.2 °C, with a rainfall average of 2,000 to 3,500 mm. The vegetation ranges from jungles, tule, and savanna, to *popal* (coastal vegetation dominated by fire flag) and mangroves (INEGI, 2017). Agriculture is the main use of 43.3% of the total surface of the municipality. The predominant crops include corn, pineapple, citrus, and eucalyptus (Palma-López *et al.*, 2011).

Insect collection

Eight Moericke-type water traps (García and Cabrera, 2012) were placed on the edges of the plantation. Four of them were green and the other four were yellow. This type of trap consists of a 32-cm wide and 7-cm deep round plastic trays that hold water with detergent and sodium hypochlorite to preserve it. Small holes were made on the sides of the tray to drain excess rainwater (Peña-Martínez, 1992a). The traps were placed horizontally on a stand, at a variable height according to the development of the crop. Biological material was collected on a weekly basis and stored in ethyl alcohol (70%) for subsequent taxonomic determination.

PRSV incidence

Direct search samplings were conducted every week to identify which plants presented ringspot symptoms. Infected plants were labeled with consecutive numbers to register them in a database. The presence of the virus was confirmed using samples taken from the petiole of plants with suspected infection. The samples were analyzed using serological tests at the Colegio de Postgraduados in Montecillo, State of Mexico.

Taxonomic determination

Holman's (1974) and Peña-Martínez's (1992) taxonomic keys were used to identify the specimens. Our findings were corroborated by the group's specialist, Rebeca Peña Martínez (MSc), at the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional.

Data analysis

Species accumulation curves were determined using the Chao1 nonparametric estimator (Moreno, 2001) in order to obtain the sampling efficiency for each trap color. Subsequently, the true diversity index, order 1(¹D) was calculated using the EstimateS 9.1.0 software. This procedure allowed us to compare the diversity of aphids captured with the green and yellow traps. The similarities between species caught in traps of both colors were analyzed using the Sorensen index (Magurran, 1989; Moreno, 2001). Finally, the population fluctuation of the most abundant PRSV vector species was examined and the a graph of the progress of the disease was developed.

RESULTS AND DISCUSSION

Only 18 out of 44 samplings recorded a 40% presence of aphids. Using the Chao1 richness estimator, sampling completeness was estimated at 93.46% for green traps and 85.09% for yellow traps (Figure 1). Although completeness was higher for green traps, these failed to capture the highest species richness. The opposite occurred with yellow traps, where five species were represented by a single individual, resulting in a lower completeness value.

We collected a total of 694 individuals that belonged to 20 species from 12 genera. Seven species from 3 genera were captured with green traps and 19 species from 11 genera with yellow traps (Table 1). Cortez-Madrigal and Mora-Aguilera (2008) reported similar species richness in papaya plantations in Cárdenas, Tabasco, while Magaña-López *et al.* (2020) recorded greater richness (17 species) in Cuernavaca, Morelos.

Of the aphid richness caught with green traps, *Aphis spiraecola* (43%) (Patch, 1914) and *Lipaphis pseudobrassicae* (36%) (Davis, 1914) were the most abundant species. Meanwhile, the yellow traps showed that the most abundant species was *A. spiraecola* (81%). This result was like the findings of Magaña-López *et al.* (2020), who documented that *A. spiraecola* was the most abundant species (56%) of the total collection. Despite capturing the lowest species richness, the green traps recorded the highest diversity value according to 1D (Table 2). These data are the results of the effectiveness of both colors as attractants. The green traps caught fewer aphid species with lower population variability. Therefore, the diversity

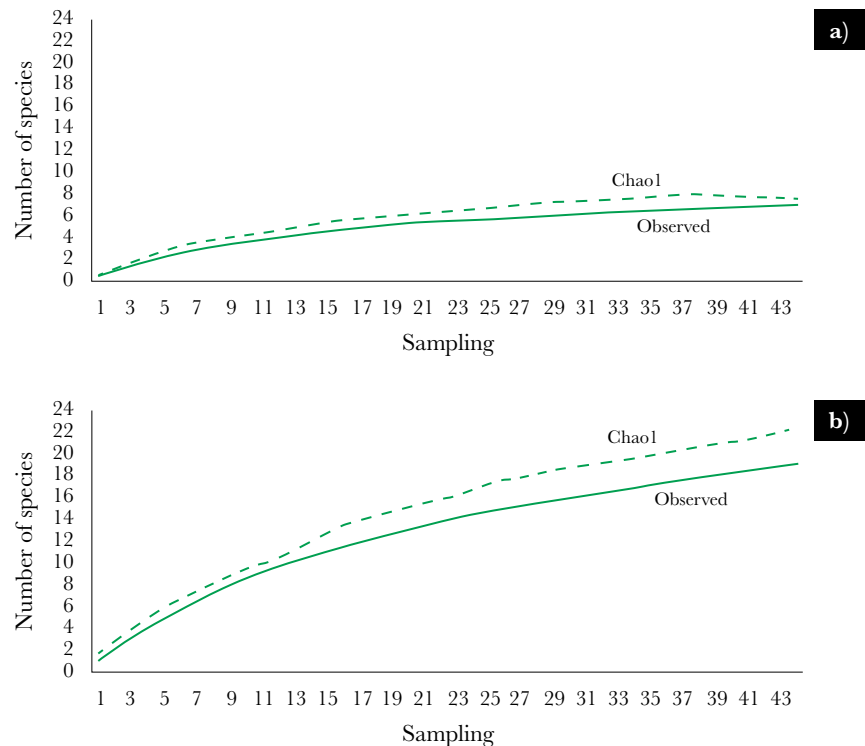


Figure 1. Accumulation curves of captured species. a) green traps. b) yellow traps.

index had a higher value. However, the highest richness was found in the yellow traps: the number of individuals in each population showed more variability, directly influencing the dominance-based diversity index and producing a lower diversity value.

In agricultural production, choosing the correct methods to evaluate populations of insect pests is crucial. As described above, an adequate evaluation guarantees a timely analysis, based on which it will be possible to conduct monitoring and propose control measures. In our study, the similarity between species captured with traps of both colors was 46%. Green and yellow traps shared six species. These data result from the low richness of species caught with green traps, which tend to be less effective for capturing insect vectors of diseases (Mensah, 1996; Hoback *et al.*, 1999; Arismendi *et al.*, 2009).

Six out of 20 aphid species captured during our study were determined to be PRSV transmitters: *Aphis aurantii* (Boyer de Fonscolombe, 1841), *Aphis gossypii* (Glover, 1877), *Aphis nerii* (Boyer de Fonscolombe, 1841), *A. spiraecola* (Patch, 1914), *Macrosiphum euphorbiae* (Thomas, 1878), and *Myzus persicae* (Sulzer, 1766). These results match the findings of Escalona (1995), who recorded *A. gossypii*, *A. nerii*, *M. persicae*, *M. euphorbiae*, and *A. aurantii* as vectors in this crop, as well as of Magaña-López *et al.* (2020), who reported the presence of *A. gossypii*, *A. nerii*, *A. spiraecola*, *M. euphorbiae*, and *M. persicae* in papaya.

A. spiraecola, *A. gossypii*, and *M. euphorbiae* were the most abundant among the transmitting species captured with both methods, with 537, 22, and 17 individuals each. Our data corroborate the conclusions of Magaña-López *et al.* (2020), who mention that these are the three most abundant species. These results also agree with the records of Cortez-Madrigal

Table 1. Aphid species captured with green and yellow traps in papaya crops in Huimanguillo, Tabasco, Mexico.

Species	Green	Yellow	Total	%
<i>Aphis aurantii</i> Boyer de Fonscolombe, 1841 *	1	1	2	0.29
<i>Aphis craccivora</i> Koch, 1854	0	9	9	1.3
<i>Aphis fabae</i> Scopoli, 1763	6	5	11	1.59
<i>Aphis gossypii</i> Glover, 1877 *	5	17	22	3.17
<i>Aphis helianthi</i> Monel, 1879	0	1	1	0.14
<i>Aphis nasturti</i> Kaltentbach, 1843	0	9	9	1.3
<i>Aphis nerii</i> Boyer de Fonscolombe, 1841*	1	3	4	0.58
<i>Aphis spiraecola</i> Patch, 1914*	31	506	537	77.38
<i>Capitophorus elaeagni</i> (del Guercio, 1894)	0	1	1	0.14
<i>Cerataphis brasiliensis</i> (Hempel, 1901)	0	1	1	0.14
<i>Geopemphigus floccosus</i> (Moreira, 1925)	0	2	2	0.29
<i>Lipaphis pseudobrassicae</i> (Davis, 1914)	26	19	45	6.48
<i>Macrosiphinum</i> sp	0	8	8	1.15
<i>Macrosiphum euphorbiae</i> (Thomas, 1878) *	0	17	17	2.45
<i>Myzus persicae</i> (Sulzer, 1766) *	0	1	1	0.14
<i>Pentalonia nigronervosa</i> Coquerel, 1859	0	11	11	1.59
<i>Rhopalosiphum maidis</i> (Fitch, 1856)	2	0	2	0.29
<i>Sarucallis kahawaluokalani</i> (Kirkaldy, 1907)	0	2	2	0.29
<i>Tetraneura nigriabdominalis</i> Sasaki, 1849	0	4	4	0.58
<i>Uroleucon ambrosiae</i> (Thomas, 1878)	0	5	5	0.72
Total	72	622	694	100

*Especies vectoras de PRSV.

Table 2. Diversity of winged aphids captured with Moericke-type water traps.

Trap	Riches	¹ D	Similarity	Chao 1	Completeness
Green	7	3.82	0.46	7.49	93.46
Yellow	19	2.80	0.46	22.33	85.09

and Mora-Aguilera (2008) in a papaya cultivation field in Tabasco. They place *A. spiraecola* as the most abundant species with 80.51% of the total aphid richness. However, our results differ from the findings of Hernández-Pérez *et al.* (2019) in plantations of Santo Domingo, Cuba, where *A. gossypii* was the most abundant species, representing 53% of the insects collected.

The six species recorded as PRSV vectors in this study fell into yellow traps, while only four of those six species fell in green traps. Based on the analysis of their population fluctuation, *A. gossypii* and *M. euphorbiae* showed a slight increase at weeks three and twelve of sampling, with 11 and 14 individuals each. Afterwards, their capture was sporadic. Likewise, *A. spiraecola* recorded two significant upturns during the study. The first peak took place in week three, when 93 individuals were captured, and the second and highest peak occurred in week ten (April), when 316 individuals were captured. After week

thirteen, captures were sporadic (Figure 2). Our data corroborate the findings of Cortez-Madrigal and Mora-Aguilera (2008) and Hernández-Pérez *et al.* (2019), who documented that March and April are the months with the highest abundance of vector species. Cortez-Madrigal and Mora-Aguilera (2008) also report that a low number of winged aphids were captured in the following months. However, our data differ from Villanueva-Jiménez and Peña-Martínez (1991) and De los Santos *et al.* (2000), who mention that the maximum abundance of winged organisms is recorded in January, February, August, and September.

The presence of PRSV was first recorded on week 7 of sampling in nine diseased plants, which amounted to an incidence of 1.3%. By week 15, diseased plants numbered 290, and by week 18, 416, with a 40% and 58% incidence, respectively. By week 31, 680 plants showed symptoms of the disease (incidence: 95%). In week 44, the incidence reached 100% (Figure 3). This progression corroborates the findings of Pushpa *et al.* (2019), who report that the

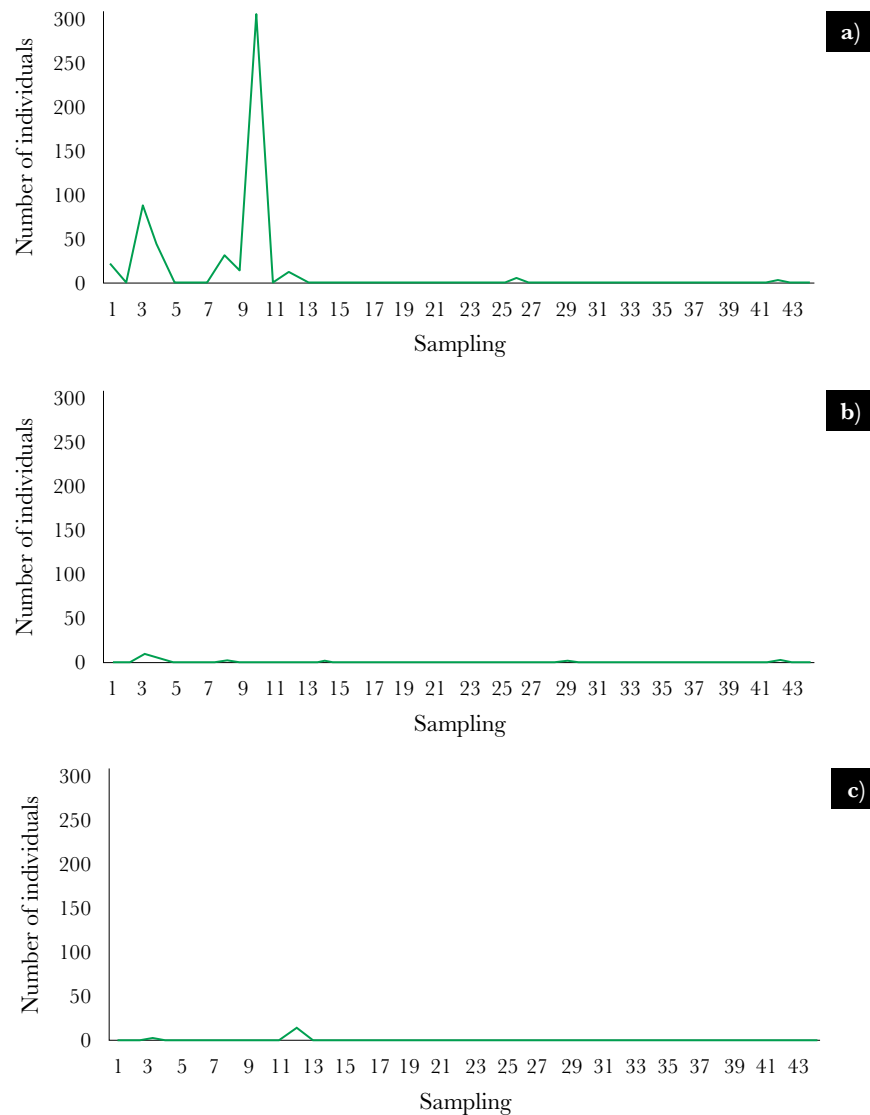


Figure 2. Population fluctuation of *A. gossypii* and *M. euphorbiae* with a slight increase in their capture in weeks three and twelve.

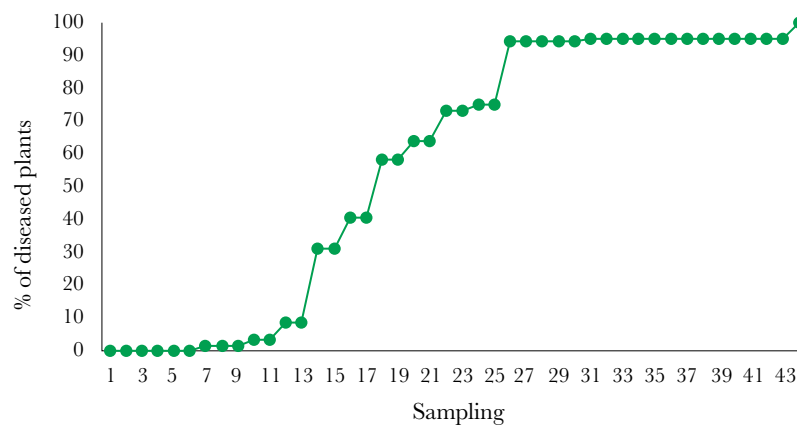


Figure 3. Cumulative incidence of PRSV symptomatic plants.

appearance of the PRSV infection is deeply related to the increase in winged aphids. In our study, the disease spread along with the population of *A. spiraecola*. However, the timing of the full infection is different: Pushpa *et al.* found that the infection had spread to all plants by week 23. Although the capture of *A. spiraecola* decreased considerably after week 13, the incidence of PRSV increased gradually over the course of the following weeks. These data are like the findings of Cortez-Madriral and Mora-Aguilera (2008), who reported the appearance of the disease, despite the decrease in captured winged aphids. The behaviour of the aphids can explain these results: aphids are non-persistent insects, the papaya plant is not their permanent host, they do not reproduce on the plant, and their stay is transitory (Vázquez *et al.*, 2010). Instead, aphids take shelter in plants growing in the surrounding area; those plants may contribute themselves to the spread of the virus (Pushpa *et al.*, 2019). Aphid behavior —along with short-distance migrations of these insects within the papaya field (Cortez-Madriral and Mora-Aguilera, 2008)— could have prevented the capture of organisms in the traps placed on the edges.

CONCLUSIONS

The correct choice of methods for capturing and monitoring insect populations in crops is essential for an adequate diagnosis and for effective control methods. The PRSV-transmitting aphids in the region of Tabasco are most abundant in the month of April. Based on its abundance, we can infer that *A. spiraecola* is the main vector of PRSV. Despite the reduction of specimens captured after the maximum population peak, the number of diseased plants increases, possibly as a result of the migrations of winged individuals within the plantation. Therefore, researching the behavior and dynamics of aphids inside the papaya crop and the surrounding areas is crucial. Such research will help to develop prevention and containment strategies for aphid as disease vectors.

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Yield evaluation of rocoto pepper (*Capsicum pubescens* R and P) with application of calcium carbonate in greenhouses

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ABSTRACT

Objective: To evaluate if the application of calcium carbonate on the soil and to the leaf influences the weight and number of fruits in rocoto pepper grown under greenhouse conditions.

Design/Methodology/Approach: The study was carried out in the greenhouse of the Facultad de Ciencias Agrotecnológicas, of the Universidad Autónoma de Chihuahua. The Taguchi method was used to develop the 13 treatments, with two factors, five levels per factor, and five repetitions per treatment, using 65 plants under study. Data was analyzed using the quadratic response surface technique, fitting the surface to determine factor levels for optimal response.

Results: Reducing soil CaCO₃ by 9% and increasing leaf CaCO₃ by 100% was necessary to obtain the highest weight in the three harvests (234.8 g).

Findings/Conclusions: A rise in the number of rocoto peppers (from 59 to 70, in the three harvest periods) required an increase in the soil and foliar CaCO₃ by 8.5% and 100%, respectively.

Keywords: Taguchi L13, response surface, Factors and Levels.

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INTRODUCTION

The rocoto pepper (*Capsicum pubescens* R and P) is native to South America. It is characterized by a fleshy pulp and is very popular in Latin American cuisine. It thrives in cool places and at altitudes from 1,700 to 2,400 m. In Mexico it can be found in high, temperate, and cold areas of Michoacán, Querétaro, the State of Mexico, Puebla, and Chiapas (Pérez and Castro, 1998). It is sown in greenhouses and the open field, using different technological components. High-tech production records high yields with high quality in an environmentally friendly arrangement (Salazar *et al.*, 2012).

The demand for rocoto pepper has increased in the last ten years. This phenomenon has led to an increase in the quality and supply of the fruits throughout the year and has motivated the development of intensive greenhouse production systems to increase their quantity and quality (Pérez, 2002). In Mexico, production is carried out mainly from July to December, which results in an absolute shortage from January to June (Gasca, 2011). The optimum temperature for the development of the rocoto pepper is 18 to 22 °C during the day and 10 to 12 °C at night. Temperatures higher than 35 °C pose a risk of flower bud abortion. Another important factor for development is relative humidity: less than 40% humidity causes pollen dehydration, which results in low pollination and low seed formation (Pérez, 2002).

Calcium carbonate is obtained by grinding and micronizing limestone and is a by-product of cement production. It may contain calcium and magnesium, usually as oxides, carbonates, or hydroxides. The main use of calcium carbonate in agriculture is to “raise the pH of acid soils and reduce the aluminum (Al) concentration in the soil solution; poor crop growth in acid soils is mainly caused by soluble Al, which is toxic to the root system of many plants”. (IPNI, 2019). Therefore, Osorio (2013) proposes the use of calcareous amendments to provide calcium to plants, favoring the production of crops, particularly those that have a high demand for Ca^{2+} and Mg^{2+} .

The amendments can contribute to the increase of calcium, magnesium, potassium, phosphorus, and sulfur in the soil, improving crop production. Calcium carbonate can be applied to the soil as a fertilizer, meeting the post-emergence calcium demand of crops. Meanwhile, soil fertilization can be complemented with foliar applications.

The low rainfall in the arid regions of northern Mexico causes a high level of calcium carbonate (from both calcareous and non-calcareous origin) in the soil. However, the high content of sodium ions—whose excessive presence displaces calcium and magnesium—in the irrigation water has affected the permeability and has caused infiltration problems in the soil. These soils are also characterized by high salinity and sodicity, which limits agricultural production in arid and semi-arid regions. These factors have increased because of an inadequate soil and irrigation water management (Schoups *et al.*, 2005; Corwin *et al.*, 2007; Li *et al.*, 2007).

In the state of Chihuahua, Mexico, peppers of the jalapeño, serrano, chilaca, and cayenne varieties are currently grown, while the rocoto pepper may be an option for greenhouse cultivation. The objective of the present work was to evaluate if the soil and leaf application of calcium carbonate (product obtained from the Chihuahua cement company) influences the weight and number of the rocoto pepper fruits under greenhouse conditions.

MATERIALS AND METHODS

The work was carried out with rocoto pepper plants that had already been established in sandy substrates in the greenhouse of the Facultad de Ciencias Agrotecnológicas of the Universidad Autónoma de Chihuahua. The greenhouse has an area of 760 m² and is equipped with a wet wall, extractors, heating, and a semi-automatic drip irrigation system. The rocoto pepper plants used had already been established in 40×40 cm black polyethylene bags, with a capacity of 16 L⁻¹ (equivalent to 20 kg of sand substrate). The rows in which the bags with the substrate were placed were 50 cm apart from each other and included a 90-cm alley. For their part, the plants were 30 cm apart from each other. We used a drip irrigation system and applied the nutrient solution (fertigation) on a daily basis (Table 1).

Table 1. Amount of nutrients applied to the rocoto pepper crop.

N	P	K	Ca	Mg	Fe	Mn	Zn	B	Cu
Mg L^{-1}									
305	54	400	164	54	2	1	1	0.50	0.05

A Taguchi method with two factors and five levels per factor was established for the study, generating 13 treatments with five repetitions per treatment and using 65 experimental plants (each experimental unit consisted of one plant) (Table 2). Given the factorial nature of the Taguchi method employed, a complete quadratic response surface analysis was performed, fitting the surface to determine the factor levels for an optimal response (SAS, 1989).

The response variables were the fruit weight (g) and the number of fruits. The former was measured with a digital scale; the fruit weight of each treatment and each repetition was measured to obtain the average. The latter variable was determined by physical counting at the end of each harvest, while the fruit weight was measured.

RESULTS AND DISCUSSION

The average weight of the rocoto pepper fruits obtained in the three harvests was 195.5 g, while 12% of the fruits obtained an average weight of 209 to 234.8 g during the same period. This result indicates that the highest weight requires the application of 2,928.7 kg ha⁻¹ of CaCO₃ to the soil and 26 L ha⁻¹ of CaCO₃ on the leaves (Table 3).

Calcium is essential to maintain some physical properties of the horticultural products, including firmness, which is an important quality characteristic. Excessive softening is one of the main factors that reduces quality and limits the commercialization of fresh products (Pablo *et al.*, 2010). Another quality factor is weight, which is associated with decay during the post-harvest handling of the fruit (González *et al.*, 2009). Products treated with calcium lose less weight, because pectin allows calcium ions to increase the stability of cell walls; although plant cell walls are permeable to water, the disassembly of this structure is not reduced. The resistance of this structure to the water flow is increased (Pablo *et al.*, 2010).

The number of fruits in the three harvest periods had a range of 59 to 70 fruits (a 19% increase). Achieving that gain requires an increase in the amounts of CaCO₃ in the soil and the leaves, in a 9% and 100% rate, respectively (Table 4). Therefore, achieving the highest response in the number of peppers (70) requires an increase of CaCO₃ in the soil and in the leaves of 3,479.1 kg ha⁻¹ and 25.6 L ha⁻¹, respectively. Valerio and Molina (2012) reported a significant response in rice yield with the application of Cal 56. Sánchez and Durand (2002) reported that Ca nitrate at 6% significantly increased the number of fruits in three mango cultivars, when Ca nitrate was combined with boron and applied to the soil after flowering.

CONCLUSIONS

Leaf applications of calcium carbonate during plant development improved the weight and number of rocoto peppers from 12% to 19%. Reducing the amount of CaCO₃ in the soil by 9% and raising the leaf CaCO₃ up to 100% increases the weight of the rocoto pepper by 12%. Increasing the number of peppers by 19% requires the application 9% and 100% of CaCO₃ to the soil and the leaves, respectively.

Table 2. Factors and levels for leaf and soil applications on rocoto pepper.

Factors	Levels				
	1	2	3	4	5
Calcium Oxide (CaO) kg ha ⁻¹ (Soil)	0.0	800	1600	3200	6400
Calcium Carbonate (CaCO ₃) kg /1000 L ⁻¹ (Foliar)	0.0	4	8	16	32

The fertilizers used in the preparation of the nutrient solution were calcium nitrate (N 15.5%, Ca 19.0%), potassium nitrate (N 12.0%, K 45.0%), ultrasol MAP (N 12.0%, P 61.0%), sulfate magnesium (Mg 9.82%), solubor (B 20.5%), copper sulfate (Cu 25.5%), ferrous sulfate (Fe 20.0%), tradecorp (Mn 13.0%), zinc sulfate (Zn 36.0%). The content of the calcium carbonate used (CaCO₃; 99.8% purity, 59.52% CaO, 7.44% SiO₂, 2.09% Al₂O₃, 0.12% Na₂O, 0.09% Fe₂O₃).

Table 3. Maximum response surface¹ for weight in three rocoto pepper harvests under greenhouse conditions, with soil and leaves treated with calcium carbonate.

Regression		Weight	
		Factors	
		CaCO ₃ Soil	CaCO ₃ Foliar
		0.2055 ^W	0.1552
Linear (L)	0.0956 ^W		
Cuadratic (C)	0.6345		
Products	0.1513		
Model	0.1876	R ² 0.5943 C.V. 13.46	μ 195.5
Weight g	E.E. ^Z	Kg ha ⁻¹	L ha ⁻¹
209.0	16.8	3200.0	12.8
211.0	17.1	3357.1	13.9
213.0	17.2	3423.0	15.2
215.0	17.1	3421.9	16.5
217.2	17.0	3383.7	17.9
219.5	16.9	3325.9	19.2
222.1	16.9	3256.2	20.5
224.9	17.2	3179.8	21.8
228.0	17.9	3098.9	23.0
231.3	19.1	3014.9	24.3
234.8	20.8	2928.7	25.6
Predicted fixed point		Critical values decoded	
209.78		4880.3	8.59

^XRidge analysis; μ Overall mean, C.V. Coefficient of variation, R² Coefficient of determination. ^W Probability of F: Pr ≥ 0.05 Not significant, Significant 0.05 ≤ Pr ≤ 0.01, highly significant Pr ≤ 0.01. ^Y significant linear (L), quadratic (C) response (Pr > |t|) significant products of that nutrient with the rest; ^Z standard error.

Table 4. Maximum response surface¹ for number of peppers in three cumulative harvests under greenhouse conditions, with soil and leaves treated with calcium carbonate.

Regressión		Number of chillies	
		Factors	
		CaCO ₃ Soil	CaCO ₃ Foliar
		0.4083	0.3029
Linear (L)	0.1310		
Cuadratic (C)	0.5602		
Products	0.5598		
Model	0.3215	R ² 0.5052 C.V. 16.16	μ 57.0
Number of chillies	E.E. ^Z	Kg ha ⁻¹	L ha ⁻¹
59	5.89	3200.0	12.8
60	6.00	3384.4	13.8
61	6.04	3488.1	15.1
61	6.04	3537.8	16.4
62	6.01	3557.5	17.7
64	6.00	3560.6	19.0
65	6.01	3553.6	20.3
66	6.14	3540.3	21.7
68	6.40	3522.7	23.0
69	6.86	3502.0	24.3
70	7.53	3479.1	25.6
Predicted fixed point		Critical values decoded	
60		4882.5	9.16

^xRidge analysis; μ Overall mean, C.V. Coefficient of variation, R² Coefficient of determination. ^wProbability of F: Pr \geq 0.05 Not significant, Significant 0.05 \leq Pr \leq 0.01, highly significant Pr \leq 0.01. ^y significant linear (L), quadratic (C) response (Pr $>$ |t|), significant products of that nutrient with the rest; ^Zstandard error.

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Interaction between nitrogen doses and alfalfa (*Medicago sativa* L.) incorporation in lettuce (*Lactuca sativa* L.) production

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ABSTRACT

Objective: To determine the optimal nitrogen dose combined with alfalfa in the growth, yield, and ion concentration in the sap of the lettuce leaf.

Design/Methodology/Approach: We used a completely randomized experimental design, with a 2×5 factorial arrangement and nine repetitions in each treatment. The treatments consisted of five nitrogen doses (200, 250, 300, 350, and 400 kg ha⁻¹) and two soil conditions (with and without alfalfa).

Results: Aerial fresh weight (AFW), aerial dry weight (ADW), end-to-end diameter of the shoot (EDS), maximum diameter of the shoot (MDS), shoot weight (SW), and yield were higher when a 200-300 kg ha⁻¹ dose of N was applied along with alfalfa. This application had a similar effect to the 300 kg dose of N ha⁻¹, applied without the addition of alfalfa. The concentration of nitrates (NO₃⁻) in the sap decreased with the addition of alfalfa and 200 kg of N ha⁻¹. The concentration of potassium (K⁺) was higher with or without the addition of alfalfa and 250 kg of N ha⁻¹. The concentration of calcium (Ca²⁺) was higher in the plants that received a 400-kg dose of N ha⁻¹, whether alfalfa was included or not.

Study Limitations/Implications: The lack of equipment prevented the determination of the nitrogen available in the soil.

Findings/Conclusions: The incorporation of alfalfa into the soil is a good alternative to improve lettuce production and reduce the use of nitrogen fertilizers.

Keywords: Growth, quality, yield, nitrate reduction.

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INTRODUCTION

Nitrogen (N) is an essential macronutrient for the growth and development of plants. It is part of amino acids, nucleic acids, chlorophyll, amino enzymes, and alkaloids. In addition, it is involved in the cell multiplication and differentiation processes (Balta *et al.*, 2015; Rodríguez *et al.*, 2020). However, the excessive use of nitrogen fertilizers is directly related to increased production costs and environmental pollution problems; therefore, it is essential to have information that helps to determine the adequate amounts needed to obtain maximum crop yields (Rodríguez *et al.*, 2020). Nitrogen fertilization in the

Mexican agricultural sector is related to a major source of greenhouse gas emissions. The said fertilization emits nitrous oxide and contributes 12.3% of the total greenhouse gas emissions (González and Camacho, 2017).

One of the alternatives to reduce the use of nitrogen fertilizers is the rotation of nitrogen-fixing species (Fabaceae) with the horticultural crop of interest. Alfalfa (*Medicago sativa* L.) (Fabaceae) can fix atmospheric nitrogen (N_2) through a symbiotic association with bacteria of the *Rhizobium* genus, converting this element to a form that the plants can assimilate (Delgado, 2015; Guzmán and Montero, 2021). It plays an important role in agriculture, as a consequence of its contribution to the reduction of the use of fossil energy and the emission of greenhouse gases produced by the use of nitrogen fertilizers (Ángeles and Cruz, 2015). Biological nitrogen fixation makes an important contribution to the N in the soil (Celaya and Castellanos, 2011). The incorporation of Fabaceae residues into the soil can increase its N content (Magaña *et al.*, 2020). In addition, the use of this type of plants as green manure in horticultural crops supplies the N that the plant demands, favoring the reduction of the excessive use of fertilizers, improving fertility, and reducing soil erosion (Castro *et al.*, 2018; Magaña *et al.*, 2020). This process contributes to the mitigation of environmental impact. Some studies report that the N provided by alfalfa is usually enough to achieve the optimal yield of some Poaceae (grasses) in the first cycle; however, sometimes the demand of the plant can also be satisfied through minimum amounts of nitrogen fertilizer. In addition to the N provided by alfalfa, previous studies have shown that soil structure can also be improved (Yost *et al.*, 2014). The process of biological nitrogen fixation in alfalfa through symbiosis could increase from 24 to more than 584 kg of N ha⁻¹ year⁻¹ and, in some cases, supply up to 90% of the plant's requirements (Ángeles and Cruz, 2015).

For its part, lettuce is one of the most consumed vegetables worldwide, given its low level of calories, sodium, and fat. Likewise, it is an excellent source of fiber, iron, folic acid, and vitamin C (Kim *et al.*, 2016). Therefore, our objective was to determine which nitrogen dose (combined with the incorporation of alfalfa) is optimal to improve growth, yield, and the ion concentration in the sap of the lettuce leaf.

MATERIALS AND METHODS

The study was carried out from August 2019 to September 2021 in the Campo Experimental of the Departamento de Horticultura de la Universidad Autónoma Agraria Antonio Narro, in Saltillo, Coahuila, Mexico (25° 21' 22.5" N, 101° 02' 08.7" W), located at an altitude of 1,610 m. The study site has a loamy soil, with an apparent density of 1.03 g cm⁻³ and a pH of 7.94. The average annual temperature is 16.8 °C and the climate is dry and semi-arid. In August 2019, alfalfa seeds cv. Giant were sown, in previously prepared 80-cm wide beds. The sowing density was 30 kg ha⁻¹ of seed, and a broadcast sowing method was used. A drip irrigation system was installed, using a Toro[®] Aqua-Traxx[™] tape of 6,000 calibers, with 20 cm between the emitters and a hydraulic flow rate of 1.05 L h⁻¹. The plants were irrigated 3 h per day, twice a week, and no fertilization was applied during the cycle of this crop. The last three alfalfa cuts were incorporated by hand into the beds where the lettuce seedlings were subsequently transplanted and covered with black polyethylene plastic mulch (1.20 m wide, 90 μm caliber, TACSA[®]).

Vegetal material

Romaine lettuce seedlings var. Capitata were used. This variety forms a tight shoot through the inner leaves and has wide and sinuous leaves. Its growth cycle lasts for approximately 60 d. The transplant was carried out on July 20, 2021, with seedlings that had five leaves. The distance between plants was 30 cm and the rows were established 80 cm apart from each other. Consequently, the planting density was adjusted to 50,000 plants ha⁻¹.

Treatments

The treatments consisted of five N doses (200, 250, 300, 350, and 400 kg ha⁻¹) and two soil conditions (with and without alfalfa). Their combination resulted in a total of 10 treatments. A 150 kg ha⁻¹ dose of phosphorus (P) and a 250 kg ha⁻¹ dose of potassium (K) were applied to each treatment. The source of those nutrients was: ammonium sulfate (20.5-00-00-24), monoammonium phosphate (12-61-0), and potassium nitrate (12-00-45). The fertilization dose was divided according to the phenological stage of the crop. The fertilization for each treatment began seven days after the transplant and it was carried out twice a week. The irrigation frequency depended on the water requirements of the plants, but on average it was carried out for 2 h every two days. The experimental design used was completely randomized with a 2×5 factorial arrangement and each treatment consisted of nine repetitions.

Parameter determination

The variables were measured on September 30, 2021, at 70 days after transplant (dat). To determine the aerial fresh weight (AFW), the aerial part was separated from the plant and weighed with a Rhino[®] 0.01 precision electronic scale. The outer leaves were removed, leaving only the edible part, to determine shoot weight (SW), using the said scale. The end-to-end diameter of the shoot (EDS) and maximum diameter of the shoot (MDS) were determined with an Insize[®] 1205-3002S vernier calliper. Subsequently, the aerial part of the plant was put in brown paper bags and then placed in a Blue M[®] POM-246F[™] drying oven at a temperature of 65 °C for 72 h. Finally, the aerial dry weight (ADW) was recorded. To determine the ion concentration of nitrate (NO₃⁻), potassium (K⁺), and calcium (Ca²⁺), three plants were selected per repetition in each treatment. The samples were collected from 10:00 to 11:00 am and, once they were properly identified, they were stored in a cooler. The samples were a combination of the external and internal part of the lettuce. The ions from the petiole cell extract were determined with the Horiba[®] LAQUAtwin[™]. The yield (t ha⁻¹) was obtained multiplying the SW by the planting density (50,000 plants ha⁻¹).

Statistical analysis

The data obtained were subjected to an analysis of variance (ANOVA) and the mean comparison was carried out with Tukey's test (α of $p \leq 0.05$), which was carried out with the 2008 version of the InfoStat statistical software.

RESULTS AND DISCUSSION

Aerial fresh weight (AFW), aerial dry weight (ADW), shoot weight (SW), end-to-end diameter shoot (EDS), and maximum diameter shoot (MDS) were significantly affected by the addition of the amendment. These same parameters were significantly affected by different N doses, as well as by the interaction of both factors, except for the EDS (Table 1). The plants developed in the soil to which alfalfa had been added showed higher parameters than those developed on the soil without this amendment (Table 1). The highest AFW, ADW, SW, and MDS were obtained with the 300-kg dose of N ha⁻¹. These variables showed a decrease with higher and lower doses, while the EDS was higher with 250 kg of N ha⁻¹ than with the higher and lower doses applied (Table 1).

Both the concentrations of nitrate (NO₃⁻) and calcium (Ca²⁺) ions in the lettuce sap, as well as the yield (ha⁻¹) were significantly affected by the addition of alfalfa. However, potassium (K⁺) did not show a significant difference (Table 2). In these same parameters, significant differences were recorded with the application of different doses of N. Likewise, these variables were influenced by the interaction between the two factors (Table 2). The highest NO₃⁻ concentration was recorded in the plants which received an application of 400 kg of N ha⁻¹, while those to which doses of 200, 250, and 350 kg of N ha⁻¹ were applied showed a decrease in the concentration of the said ion. The lowest NO₃⁻ concentration was obtained with the 300-kg dose of N ha⁻¹. The Ca²⁺ concentration was higher when 400 kg of N ha⁻¹ were added, but it decreased when a 200-350 kg dose of N ha⁻¹ was applied. Regarding K⁺, higher concentrations were

Table 1. Effect of the amendment (alfalfa) and N addition in the soil on the aerial fresh weight (AFW/PFA), aerial dry weight (ADW/PSA), shoot weight (SW/PC), end-to-end diameter shoot (EDS/DPC), and maximum diameter shoot (MDS/DEC) of romaine lettuce var. Capitata.

Alfalfa	AFW (g plant ⁻¹)	ADW (g plant ⁻¹)	SW (g plant ⁻¹)	EDS (cm)	MDS (cm)
With alfalfa	1144.86a	38.66a	777.99a	13.87a	16.47a
Without alfalfa	752.93b	31.55b	430.21b	10.20b	12.32b
Anova p≤	0.0001	0.0001	0.0001	0.0001	0.0001
N (kg ha ⁻¹)					
200	854.67c	36.77b	520.46b	11.82abc	14.03b
250	1001.55b	34.86b	623.11ab	12.72a	14.64ab
300	1164.94a	41.56a	741.91a	12.65ab	15.40a
350	868.88c	34.31b	624.67ab	11.69bc	14.30ab
400	854.43c	28.05c	510.33b	11.29c	13.60b
Anova p≤	0.0001	0.0001	0.0001	0.0003	0.0058
Interaction p≤	0.0001	0.0003	0.0171	0.3283	0.0086
CV (%)	8.49	11.39	19.50	7.15	8.04

≤ 0.01 and 0.05 = Significant. ≥ 0.05 Not Significant. Anova = analysis of variance. Interaction = Alfalfa×N. CV = coefficient of variation. Means with different letters indicate significant effects, according to Tukey's multiple comparison test (p≤0.05). Aerial fresh weight (AFW), aerial dry weight (ADW), end-to-end diameter of the shoot (EDS), maximum diameter of the shoot (MDS), shoot weight (SW).

recorded with the 250-kg dose of N ha⁻¹, while the concentration of the ion decreased with other doses (Table 2).

The yield was higher with 300 kg of N ha⁻¹; however, adding higher and lower doses resulted in a lower parameter (Table 2). Overall, most of the parameters evaluated in this research were higher in plants grown in soil with alfalfa than in soils grown without alfalfa.

The AFW was higher when 200 kg of N ha⁻¹ were applied, and alfalfa was added to the soil. As the N dose increased, the AFW slightly decreased. The plants to which only chemical fertilization was applied recorded the maximum AFW with the 300-kg dose of N ha⁻¹; meanwhile, higher and lower doses showed a decrease in the said variable (Figure 1). A similar behavior was observed in the ADW (Figure 2). Overall, plants that grew in soil to which alfalfa had been added showed higher values than those that were nurtured only with nitrogen fertilization. The increases in the AFW and ADW of the plants developed with alfalfa may be caused by the greater availability of N in the soil to which alfalfa had been added. Magaña *et al.* (2020) and other authors indicate that the N content in the soil can be increased through the incorporation of Fabaceae residues. This measure can diminish nitrogen fertilization in certain crops, such as corn (Cela *et al.*, 2011). In addition, N favors the growth and development of plants, given its structural function and its involvement in cell multiplication and differentiation (Rodríguez *et al.*, 2020). For their part, the diminishing of the AFW and ADW of the plants that developed only with nitrogen fertilization (200-250 kg doses of N ha⁻¹) are likely the result of the insufficient amount of N added for the achievement of an adequate growth, which decreases the productive potential (Rodríguez *et al.*, 2020). Martínez *et al.* (2008) reported considerable reductions

Table 2. Effect of the amendment (alfalfa) and N addition in the soil on the concentration of nitrate (NO₃⁻), calcium (Ca²⁺), potassium (K⁺) ions in the sap and yield of romaine lettuce var. Capitata.

Alfalfa	NO ₃ ⁻ (mg kg ⁻¹)	Ca ²⁺ (mg kg ⁻¹)	K ⁺ (mg kg ⁻¹)	Yield (t ha ⁻¹)
with alfalfa	3176.67b	156.60a	1230.00a	38.90a
without alfalfa	6280.00a	122.00b	1258.33a	21.51b
Anova p≤	0.001	0.001	0.180	0.001
N (kg ha ⁻¹)				
200	4800.00b	145.00b	1250.00b	26.02b
250	4550.00b	124.25c	1442.50a	31.16ab
300	3800.00c	122.50c	1158.33bc	37.10a
350	4700.00b	79.75d	1120.00c	31.23ab
400	5791.67a	225.00a	1250.00b	25.52b
Anova p≤	0.001	0.001	0.001	0.001
Interaction p≤	0.001	0.001	0.001	0.017
CV (%)	6.21	7.27	4.49	19.50

≤ 0.01 and 0.05 = Significant. ≥ 0.05 Not Significant. Anova = analysis of variance. Interaction = Alfalfa × N. CV = coefficient of variation. Means with different letters indicate significant effects according to Tukey's multiple comparison test (p≤0.05).

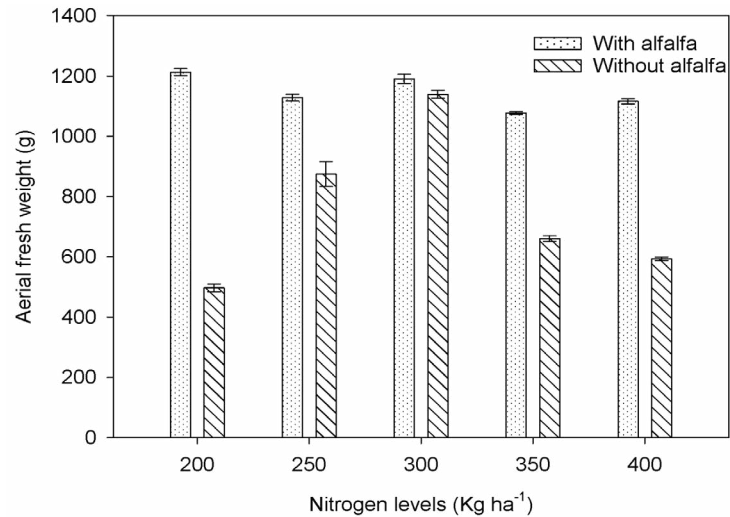


Figure 1. Effect of the interaction between alfalfa incorporation and different N doses on the aerial fresh weight (AFW) of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

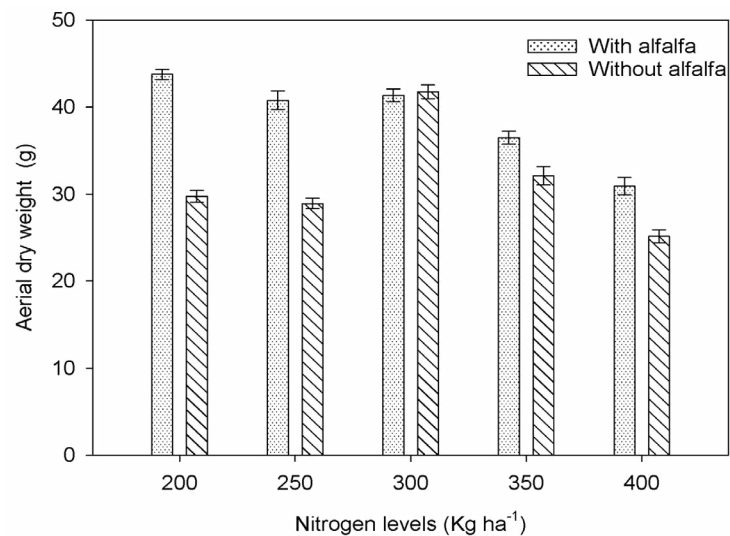


Figure 2. Effect of the interaction between alfalfa incorporation and different doses of N on the aerial dry weight (ADW) of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

in the fresh and dry weight of the cape gooseberry fruits nurtured with minimal amounts of N.

The EDS was higher (250 kg of N ha⁻¹) in plants developed on soil with alfalfa. However, as the N dose increased, the EDS tended to decrease slightly. In the case of plants developed only with nitrogen fertilization, a higher EDS was found (300 kg of N ha⁻¹). Likewise, the application of higher and lower doses resulted in a slight decrease in EDS (Figure 3). This behavior was similar in the MDS (Figure 4). The EDS and MDS reductions on plants that were nurtured only with nitrogen fertilization may be related to the amount of N applied. In this regard, Martínez *et al.* (2008) observed that applying

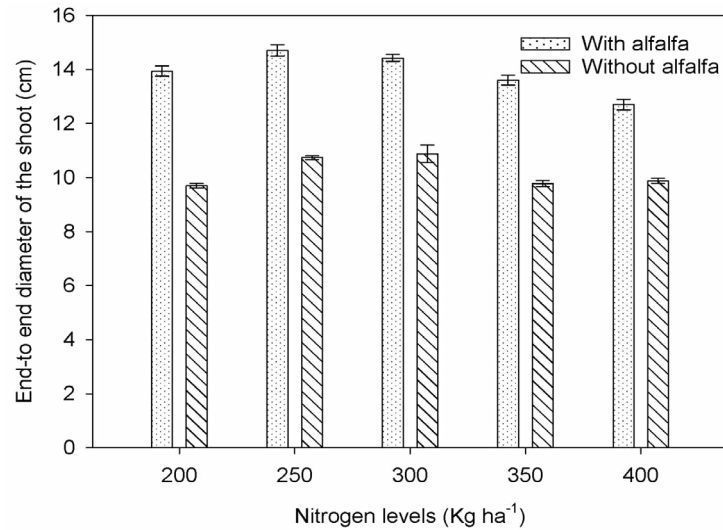


Figure 3. Effect of the interaction between alfalfa incorporation and different doses of N on the end-to end diameter of the shoot (EDS) of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

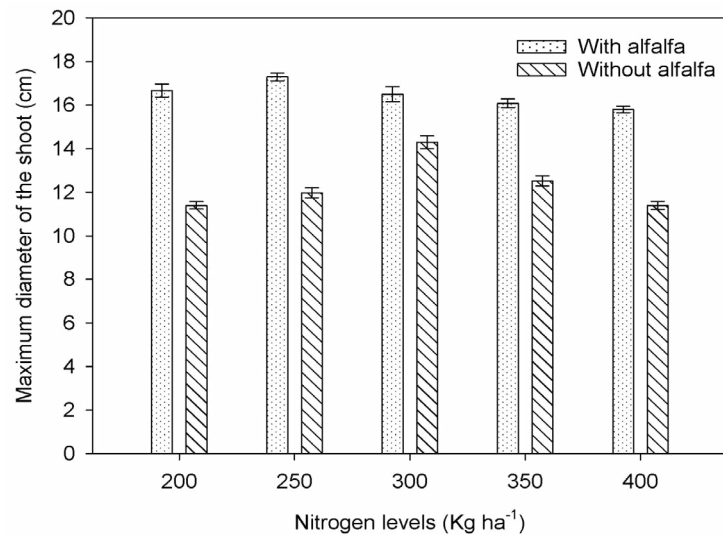


Figure 4. Effect of the interaction between alfalfa incorporation and different doses of N on the maximum diameter of the shoot (MDS) of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

minimum amounts of N during fertilization had a negative effect on the end-to-end and maximum diameter of cape gooseberries with respect to complete fertilization, indicating that they had an inadequate growth and low productivity. Likewise, the size of the lettuce showed a considerable increase when alfalfa was added.

The SW was higher in the plants that were developed in soil with alfalfa and with application of 200 and 300 kg of N ha⁻¹. However, when higher N doses were added, the SW tended to decrease. In the plants that were nurtured only with nitrogen fertilization, the highest SW was recorded with the 300-kg dose of N ha⁻¹, because the addition of

lower and higher doses led to a decrease of this parameter (Figure 5). Overall, the plants that developed in the soil to which alfalfa and different doses of N were incorporated had higher SW than those that only received nitrogen fertilization. These results could possibly be attributed to the successful supplementation by alfalfa of the N demanded by the plant. In this regard, Samaddar *et al.* (2021) indicate that alfalfa increases the availability of N in the soil and, consequently, increases crop yield. Ángeles and Cruz (2015) point out that, in some cases, alfalfa can supply up to 90% of the N requirements of the plant. The results indicate that alfalfa can help to reduce the use of nitrogen fertilizers, since maximum yields can be achieved by applying minimum amounts of N.

The lowest NO_3^- concentration in the sap was obtained with the 200-kg dose of N ha^{-1} in the plants that were developed in the soil with alfalfa. As the N dose increased, the NO_3^- concentration also tended to increase. Overall, lower NO_3^- concentrations were found in plants that were grown in soil to which alfalfa had been added than in those soils were grown without alfalfa. The accumulation of NO_3^- in plants depends on the amount of N applied (Liu *et al.*, 2014) and the size of the biomass of the plants. Ollúa *et al.* (2016) pointed out that as the N dose increases, the concentration of NO_3^- in the loose-leaf lettuce also tends to increase. These findings match the results found in this research for the plants that were developed in the soil to which alfalfa had been added. Regarding the plants that were developed only with nitrogen fertilization, a higher NO_3^- concentration was recorded when a 200-400 kg dose of N ha^{-1} was applied. However, supplying 200, 250, 300, and 350 kg doses of N ha^{-1} tended to decrease the concentration of the said ion and the lowest concentration was obtained with 300 kg of N ha^{-1} (Figure 6). This behavior can be directly related to biomass, since the plants that received a lower amount of N had lower biomass. A similar effect occurs in plants nurtured with high amounts of N: the concentration of this ion in the sap tends to be higher. However, Ollúa *et al.* (2016) point out that applying >75 kg doses of N ha^{-1} to butterhead lettuce reduces the NO_3^-

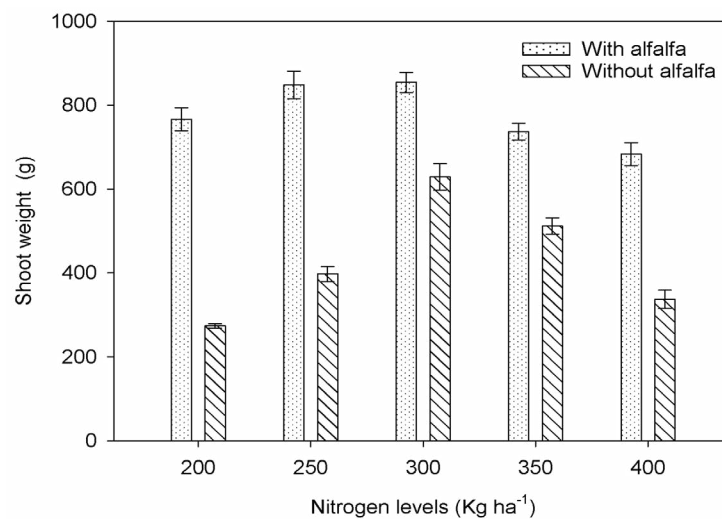


Figure 5. Effect of the interaction between alfalfa incorporation and different doses of N on the shoot weight (SW) of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

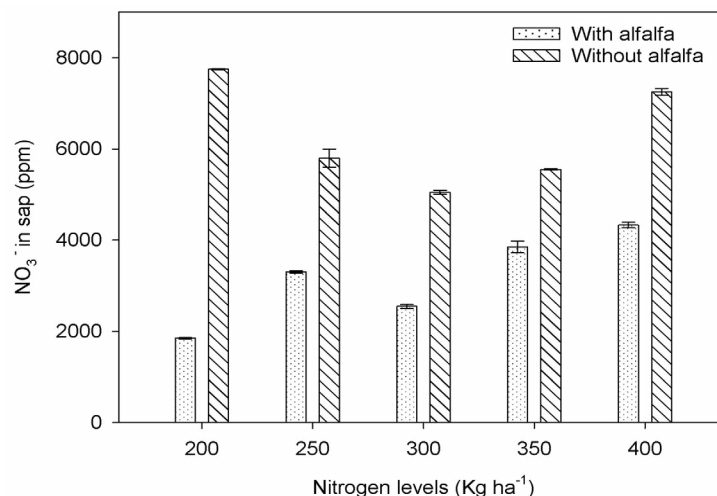


Figure 6. Effect of the interaction between alfalfa incorporation and different doses of N on the NO_3^- concentration of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

concentration in the sap. This behavior is possibly the result of a dilution effect, since the plants obtained a higher biomass.

The highest K^+ concentration was registered with the 250-kg dose of N ha^{-1} . This phenomenon was observed in the plants that developed on the ground both with and without alfalfa. Supplying higher and lower doses resulted in a slight reduction in the concentration of the said ion (Figure 7). In this regard, Gaona *et al.* (2020) report higher K^+ concentrations in sweet granadilla leaves with high doses of N, possibly as a consequence of the synergism that exists between them. However, the concentrations of this ion may have been affected by the concentration-dilution phenomenon: low and high N doses led to lower biomass, while the 300-kg dose of N ha^{-1} resulted in a higher biomass. Therefore, the concentration of said ion was negatively affected.

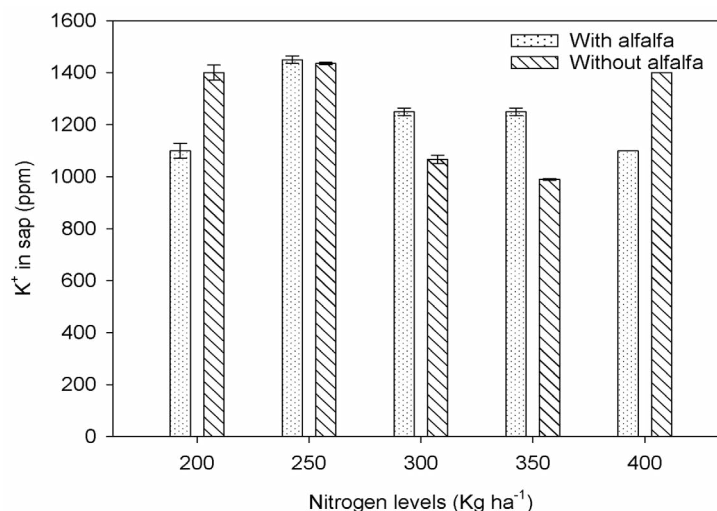


Figure 7. Effect of the interaction between alfalfa incorporation and different doses of N on the potassium (K^+) concentration of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

The Ca^{2+} concentration was higher when 400 kg of N ha^{-1} were added; this phenomenon was observed in the plants developed in soil with and without alfalfa. However, when 200 and 350 kg doses of N ha^{-1} were added, a decrease in the concentration of said ion was registered (Figure 8). Hernández and Rubilar (2012) reported reductions in the foliar concentration of Ca^{2+} in shoots of *Pinus radiata* hedges as the dose of N increased. The high Ca^{2+} concentrations resulting from the 400-kg dose of N ha^{-1} are possibly due to a concentration effect. The plants nurtured with the said dose had lower biomass, as a consequence of the high amounts of N applied.

The yield was higher with the 200- and 300-kg doses of N ha^{-1} combined with alfalfa. However, it tended to decrease when higher doses were added (Figure 9). The increases

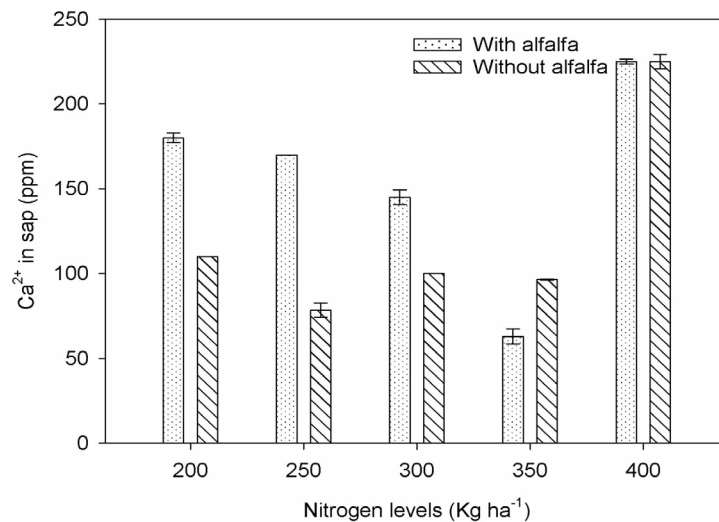


Figure 8. Effect of the interaction between alfalfa incorporation and different doses of N on the calcium (Ca^{2+}) concentration of romaine lettuce var. Capitata. The bars indicate the standard error of the means.

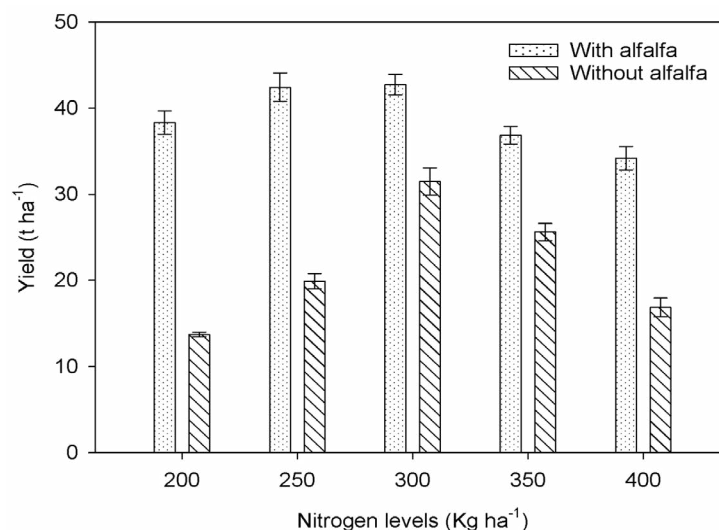


Figure 9. Effect of the interaction between alfalfa incorporation and different doses of N on the romaine lettuce var. Capitata yield. The bars indicate the standard error of the means.

in yield with the minimum doses of N may be caused by the presence of enough N for the plants in the soil, as a result of the incorporation of alfalfa and its N fixation. For their part, Cela *et al.* (2011) found high yields of corn grain with doses of 0 and 150 kg of N ha⁻¹ after using alfalfa. Their results are similar to those found in this study, which suggests that the N provided by alfalfa can supply the subsequent crop's demand for this nutrient.

The decrease of this parameter in the plants that were developed without alfalfa and with the application of 200 and 400 kg of N ha⁻¹ can be attributed to both the lack and excess of N. In this regard, García *et al.* (2021) did not find a significant difference in corn yield when 200 and 400 kg doses of N ha⁻¹ were applied; therefore, they suggest that 400 kg of N ha⁻¹ is an excessive dose and causes a low use of N by the plant. Therefore, applying large amounts of N does not necessarily increase crop yield (Sotomayor *et al.*, 2017; Rodríguez *et al.*, 2020). In this regard, Grijalva *et al.* (2016) observed a negative effect on wheat yield when a 460-kg dose of N ha⁻¹ was applied. For their part, Sanchez *et al.* (2006) indicate that the impact of excessive N applications on biomass production is more negative than when this nutrient is deficient. A similar effect was observed in this research, especially in plants that were nurtured only with nitrogen fertilization: the maximum yield was recorded with 300 kg of N ha⁻¹. Meanwhile, Grijalva *et al.* (2016) recorded a maximum wheat yield with a 230-kg dose of N ha⁻¹. For their part, Rodríguez *et al.* (2020) found higher yields with a 200-g dose of N plant⁻¹ in passion fruit cultivation, while lower doses had a negative impact on the growth and development of the plants.

CONCLUSIONS

The use of alfalfa favors growth, yield, and ion concentration in lettuce plants. The addition of alfalfa and a 200-kg dose of N ha⁻¹ decreases the NO₃⁻ concentration in the leaf sap. The Ca²⁺ concentration was higher in plants that were developed with alfalfa. The incorporation of alfalfa to the soil, combined with 200- and 300-kg doses of N ha⁻¹ increased the growth and yield of the lettuce plants. These findings suggest that the incorporation of alfalfa to the soil is a good alternative to improve lettuce production and reduce the supply of nitrogen fertilizers.

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Effect of climatic factors on the diversity and abundance of Scolytinae and Platypodinae (Coleoptera: Curculionidae) in a pine-oak forest

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ABSTRACT

Objective: The aim of this work was to evaluate the diversity and abundance of bark beetles and ambrosial beetles and their relationship with temperature and relative humidity.

Design/methodology/approach: The study was carried out in a pine-oak forest of the northeastern Sierra of the state of Puebla, where a monitoring system was established using Lindgren traps baited with frontalin, brevicomine, alpha-pinene and beta-pinene.

Results: The presence of 23 species of bark beetles and ambrosial beetles was recorded. *Gnathotrichus sulcatus* (LeConte) and *Pseudips mexicanus* (Hopkins) were the most abundant species and their presence was significantly associated with places having high relative humidity.

Limitations on study/implications: The results obtained are limited to the studied ecosystem.

Findings/conclusions: It was concluded that the abundance of the species observed was closely related with climatic factors.

Keywords: Bark beetles, environmental temperature, relative humidity, semiochemicals, temperate forest

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INTRODUCTION

The family Curculionidae Latreille (1802) is one of the most diverse group of insects, characterized by containing some of the most economically important pests of ornamental, agricultural, and forestry crops [1, 2]. This group includes the bark beetles and ambrosial beetles (Scolytinae Latreille, (1807) and Platypodinae Shuckard, (1840)). Beetles are related to diverse processes in ecosystems, including nutrient cycling [3, 4], ecological succession [5], hydrological cycles [6], and wildfires [7], whose interaction and synergies may be intensified by climate change [8].

The Scolytinae and Platypodinae subfamilies comprise more than 6,000 and 1,000 species, respectively [1, 9]. They usually share habits and hosts (Kirkendall, 1983), and generally inhabit galleries created by females, where they lay eggs and obtain food [10]. Similarly, both subfamilies play an important role in forest ecosystems, as they eliminate diseased and unfitted plants [11], which allows the incorporation of organic matter into the soil.

In Mexico, 927 species of Scolytinae and 45 species of Platypodinae have been recorded. In the State of Puebla, 167 species of Scolytinae and four species of Platypodinae have been

recorded [12]. Although there are many records of Coleoptera species in the northeastern Region of the State of Puebla, the presence of species in the subfamilies Scolytinae and Platypodinae has not been documented [13,15]. Therefore, the objective of this study was to correlate the effect of environmental factors on the diversity and abundance of species of these subfamilies in a temperate pine-oak forest in the northeastern region of the State of Puebla.

MATERIALS AND METHODS

This study was conducted in the forested area of the Tecnológico Nacional de México, campus Instituto Tecnológico Superior de Zacapoaxtla (TecNM/ITS-Zacapoaxtla) in the northeastern region of the State of Puebla ($19^{\circ} 49' 50.34''$ and $19^{\circ} 49' 48.187''$ North latitude and $-97^{\circ} 34' 9.544''$ and $-97^{\circ} 34' 22.072''$ West longitude), with an average altitude of 2140 masl (Figure 1). The climate in this location has been humid and temperate (C(m)(f)) in the last 30 years, according to records from the National Meteorological Service (SMN, 2021). The region is characterized by 46 stormy days and 254 foggy days per year; with a minimum annual precipitation of 115.2 mm, average annual precipitation of 460.2 mm and maximum annual precipitation of 1012.5 mm; the minimum annual temperature is 12.9°C , the average annual minimum temperature is 15.6°C and the maximum annual minimum temperature is 20.9°C . The vegetation corresponds to an oak-pine forest [16].



Figure 1. Location of the northeastern highlands of Puebla where this study was carried out

Samplings were performed from May 2018 to December 2019, inside the 22 hectares belonging to the Instituto Tecnológico Superior de Zacapoaxtla. Four Lindgren traps with eight funnels (BioQuip[®]) were installed randomly. The traps were placed in nonhost trees or in metallic structures at a height of 1.5 m from the ground, and 150 m separated among them. Traps were baited with Frontalin (pheromone), endo-brevicomine (pheromone), alpha-pinene and beta-pinene (kairomone) (Synergy Semiochemicals Corp[®]). To ensure an optimal level of attraction, the replacement of the attractants of traps was done every two months.

Insects were collected from the traps biweekly, the captured specimens were placed in bottles with ethyl alcohol (70%), then they were transferred to the Forestry Department of the TecNM/ITS-Zacapoaxtla. The scolytids were identified based on the taxonomic keys of Wood [1], Cibrián *et al.* [17], Camacho [18] and Armendariz *et al.* [19]. For platypodids, the taxonomic keys of Wood [20] were used. Voucher specimens were deposited in the entomological collection of TecNM / ITS-Zacapoaxtla. The climatic factors were recorded every 30 minutes with a data Logger pro 2 (Hobo, USA) installed in the center of the 22 hectares of the property. We measured the minimum temperature, average temperature, maximum temperature, minimum relative humidity, average relative humidity and maximum relative humidity.

The sampling efficiency and the abundance coverage-based species richness estimator (ACE mean) [21], was estimated with the EstimateS v 9 program [22]. This estimator has previously been used to study Coleoptera communities in forest ecosystems [23]. A total of 100 randomizations were performed to obtain the species accumulation curve, obtaining the relationship between the number of species and the month of collection [24]. The separation between the curve of the recorded species and the curve of the estimated species indicates the number of species to be recorded within the community [25].

To determine the abundance and distribution of the species captured, the individuals were counted. Using the R-project program [26], the normality of the data was verified by means of the Shapiro-Wilk test ($\alpha=0.05$), given that the data did not show normality, the Spearman test ($p=0.001$) was used to estimate the relationship of the monthly abundance of the beetles with the climatic variables.

RESULTS AND DISCUSSION

A total of 671 specimens were collected, belonging to 23 species, of which 17 belong to the subfamily Scolytinae: *Dendroctonus adjunctus* Blandford, *Dendroctonus approximatus* Dietz, *Dendroctonus mexicanus* Hopkins, *Dendroctonus valens* LeConte, *Gnathotrichus sulcatus* Wood and Bright, *Hylastes fulgidus* Blackman, *Hylastes incomptus* Blandford, *Hylastes tenius* Eichhoff, *Hylurgops longipennis* Wood and Bright, 1992, *Hylesinus* sp. (Fabricius, 1801), *Ips concinnus* Wood and Bright, *Ips emarginatus* LeConte, *Ips integer* Wood and Bright, *Pseudips mexicanus* Cognato, *Ips plastographus* Lanier, *Xyleborus* sp.1 Eichhoff and *Xyleborus* sp. 2 Eichhoff. The remaining six species belong to the subfamily Platypodinae: *Euplatypus pini* Hopkins, *Oxoplatypus* sp. Wood and four morphotypes *Platypodinae* sp. (1, 2, 3 and 4). The species accumulation curve for the TecNM/ITS-Zacapoaxtla forest, that corresponds to the proportion of total abundance of bark beetles and ambrosia beetles presented in the

sample, in relation to the total abundance of the community [27], showed that the sampling efficiency for bark beetles and ambrosia beetles was 68.2% (Figure 2). Several authors have highlighted the ecological importance of Coleoptera [28, 29], thus, in the northeastern region of Puebla, research has been developed focused on describing the biodiversity of this group [13, 14], however, bark beetles and ambrosial beetles have not been part of such regional entomological inventories, Equihua and Burgos [30] have reported for the State of Puebla the recorded species captured in this work. It should be mentioned that the sampling efficiency achieved in this study was 68.2%, as it is estimated that exhaustive sampling is achieved with proportions greater than 70%. Similarly, Pérez and collaborators [31], during February 2010 to January 2011, estimated the diversity of Scolytinae and Platypodinae in two natural areas of the State of Tabasco, Mexico; where, they recorded 7057 specimens, belonging to 46 species and 26 genera, without managing to stabilize the species accumulation curve. Possibly, other monitoring strategies are needed to capture the full diversity of these subfamilies.

In this study the highest abundance (61%) of Scolytinae and Platypodinae species were recorded from October 2018 to February 2019). *Gnathotrichus sulcatus* was the species with the highest number of captures (269), followed by *Pseudips mexicanus* (60). In Mexico, 974 species of bark beetles and ambrosial beetles have been reported, of which 929 correspond to Scolytinae and 45 to Platypodinae. Likewise, 167 species of bark beetles and ambrosial beetles have been recorded for the State of Puebla, of which 163 are from the subfamily Scolytinae and 4 are from the subfamily Platypodinae [9]. In this study, 23 species were recorded, of which the subfamily Scolytinae obtained a higher proportion with 17 species compared to Platypodinae with four species. These differences may be due to the type of bait used since there is a variation in response to baits in bark beetle species [32]. Therefore, in relation to national and state records the diversity of bark beetles and ambrosial beetles captured represents 2.4 and 13.8 %, respectively. Similar results were

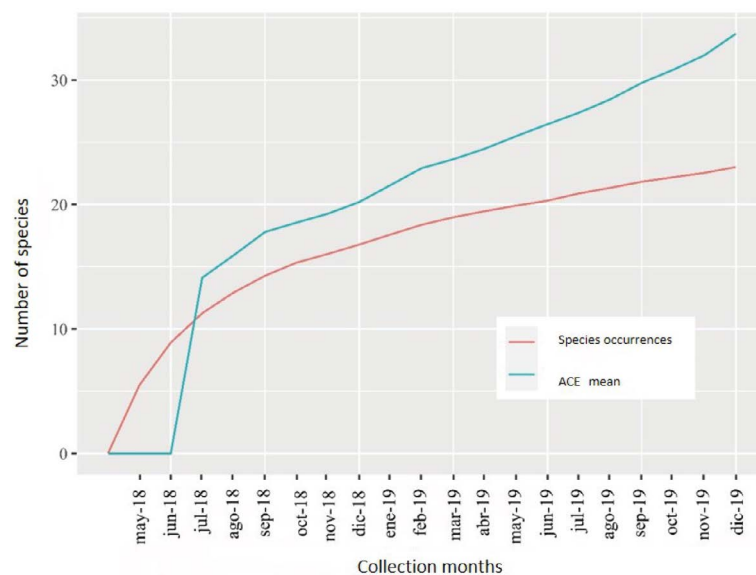


Figure 2. Species accumulation curve.

presented in a xerophytic vegetation environment in the Zapotitlán valley, Puebla [33] and matched with those results of temperate forests where richness and abundance were dominated by Scolytinae [1]. In contrast to forests, where bark beetles and ambrosial beetles are mainly species of the subfamily Platypodinae [34]. Likewise, the proportion coincided with that documented for the State of Puebla, where 97.6% of species recorded belong to the Scolytinae and 2.4% correspond to Platypodinae [9].

The average and standard deviation of the minimum temperature ($5\text{ }^{\circ}\text{C}\pm 4.4$), mean temperature ($14\text{ }^{\circ}\text{C}\pm 2.3$), maximum temperature ($28\text{ }^{\circ}\text{C}\pm 3.7$), minimum relative humidity percentage ($29\%\pm 16.6$), mean relative humidity percentage ($85\%\pm 8.6$) and maximum relative humidity percentage ($100\%\pm 0.7$) were obtained. Spearman's test showed that at least 12 species show a significant correlation between abundance and an environmental variable (Table 1). Of these, nine species showed a negative correlation with the percentage of relative humidity: *D. adjunctus*, *D. mexicanus*, *G. sulcatus*, *H. incomptus*, *I.*

Table 1. Relationships between the species of bark beetle and ambrosia beetles abundance and environmental factors, *($p < 0.001$).

	Minimum temperature (°C)	Average temperature (°C)	Maximum Temperature (°C)	Minimum relative humidity (%)	Average Relative Humidity (%)	Maximum relative humidity (%)
Scolytinae						
<i>Dendroctonus adjunctus</i>	-0.109	-0.215	0.169	0.119	-0.285	-0.583*
<i>Dendroctonus approximatus</i>	-0.512*	-0.576*	-0.064	-0.355	-0.049	0.056
<i>Dendroctonus mexicanus</i>	0.282	0.141	-0.006	0.46*	-0.143	-0.782*
<i>Dendroctonus valens</i>	0.174	0.145	0.335	0.38	0.033	0.085
<i>Gnathotrichus sulcatus</i>	0.156	-0.002	0.218	0.208	-0.336	-0.566*
<i>Hylastes fulgidus</i>	0.29	0.153	-0.417	0.247	0.08	-0.322
<i>Hylastes incomptus</i>	-0.092	-0.187	-0.061	0.041	-0.101	-0.544*
<i>Hylurgops longipennis</i>	0.148	0.077	0.105	0.284	0.133	0.017
<i>Hylastes tenius</i>	0.425	0.268	-0.18	0.459*	0.196	0.031
<i>Hylesinus</i> sp.	-0.141	-0.114	0.445	-0.247	-0.406	0.056
<i>Ips concinnus</i>	0.082	0.283	0.418	-0.31	-0.243	0.056
<i>Ips emarginatus</i>	0.333	0.148	-0.319	0.222	-0.117	-0.695*
<i>Ips integer</i>	0.246	0.143	-0.354	0.119	0.12	0.056
<i>Pseudips mexicanus</i>	0.175	-0.045	-0.06	0.218	-0.219	-0.712*
<i>Ips plastographus</i>	0.276	0.061	-0.069	0.28	-0.271	-0.84*
<i>Xyleborus</i> sp. (1)	0.256	0.095	0.063	0.253	-0.007	0.106
<i>Xyleborus</i> sp. (2)	-0.251	-0.087	0.394	-0.187	-0.325	0.074
Platypodinae						
<i>Euplatypus pini</i>	0.258	0.176	-0.097	0.429	0.184	-0.27
<i>Oxoplatypus</i> sp.	0.39	0.238	0.09	0.357	-0.328	-0.85*
<i>Platypodinae</i> sp. 1	0.19	0.242	-0.098	0.096	0.276	0.056
<i>Platypodinae</i> sp. 2	0.19	0.242	-0.098	0.096	0.276	0.056
<i>Platypodinae</i> sp. 3	0.296	0.182	-0.147	0.197	-0.314	-1*
<i>Platypodinae</i> sp. 4	-0.438	-0.486*	0.123	-0.222	-0.066	0.056

emarginatus, *I. plastographus*, *P. mexicanus*, *Oxoplatypus* sp. and *Platypodinae* sp. 3. While two species showed a positive correlation with minimum relative humidity: *D. mexicanus* and *H. tenius*. Additionally, the mean monthly temperature was negatively associated with two species: *D. approximatus* and *Platypodinae* sp. 4. Likewise, the minimum temperature was associated with one species *D. approximatus*. Of the captured species considered to be pests of economic and quarantine importance, Desmond and John [35] reported that *G. sulcatus* on the coast of British Columbia, Canada, had greater flight activity in April and May, a period that coincided with the increase of temperature, and had its maximum abundance at the beginning of May. In contrast, in this study, flight activity occurred from September to December. Data for this activity showed a negative correlation with the percentage of maximum relative humidity, according to the Spearman test, which is the factor that influences flight activity (Table 1, Figure 3).

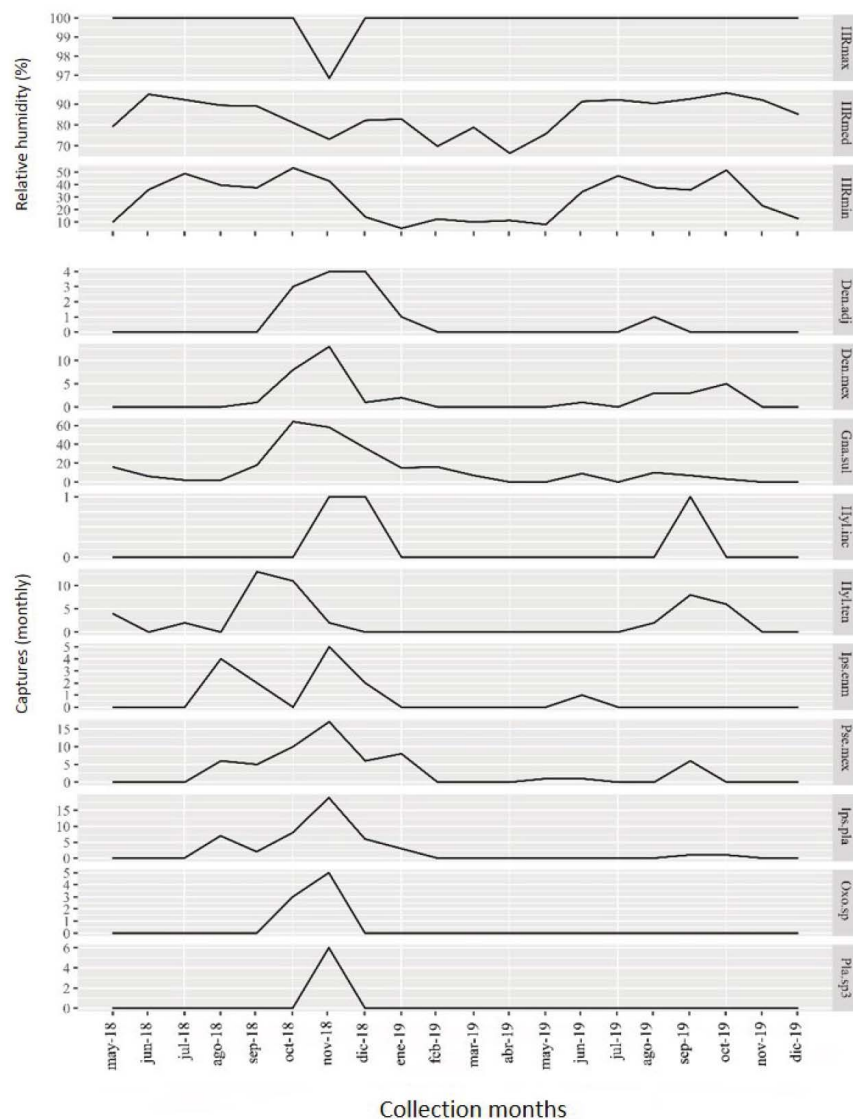


Figure 3. Relative humidity and number of bark beetle and ambrosia beetle captures.

Dendroctonus species have been considered important pests of forests. In Mexico, they are responsible for 40.5% of the area affected by pests (Pérez *et al.*, 2013). In Cofre de Perote National Park, *D. adjunctus* had the greatest increased by April, with a population decreased in August [36]. This data contrast with that reported for the state of Mexico [36], where the greatest population increased occurred during the months of August to December, a period where temperatures also declined. On the other hand, in the Aguascalientes state, *D. mexicanus* showed that, during the period from April 2015 to March 2016, the population increased was directly related to temperature [37], a difference from what was observed in this study, where temperature did not show a significant effect and minimum relative humidity was positively correlated.

During the sampling period, there was a decrease in temperature from December 2018, reaching in January 2019 the minimum temperature of -3.6°C ; these records are similar to the minimum temperature of -3°C provided by the National Commission for the Knowledge and Use of Biodiversity (CONABIO) for the area, and this decrease in temperature coincides with the decrease in insect captures. Low temperatures are one of the factors that cause a decrease of the populations of bark beetles and ambrosial beetles [38]. In Colorado, USA, temperatures below -30°C killed most of the adults and larvae of *Dendroctonus engelmanni* Hopk. [39]. In this study, the Sperman test showed that the minimum temperature was negatively correlated with *D. approximatus*, which is not considered an important pest in Mexico [40]. In turn, the minimum temperature was not correlated with species considered pests for Mexico, a similar case was reported for *D. mexicanus* in the state of Hidalgo, Mexico, where the average minimum temperature was 4°C , which did not affect the population decreased of *D. mexicanus* [41].

Our results show that environmental factors have a differential influence on the different species of ambrosial beetles and bark beetles. For example, while some species were favored by relative humidity or temperature, other species were affected by these environmental factors, affecting their population abundance. Similarly, other beetle species detected had apparently no association or response either to temperature nor relative humidity.

On a large scale, climatic factors influence the distribution of species [42], in the case of this study we were able to identify the relationship between environmental factors and the abundance of bark beetles and ambrosial beetles. In contrast, Hernández and Obregón [44], obtained non-significant data between average temperature and the abundance of bark beetles in a pine forest in the municipality of Zimapán, Hidalgo. This discrepancy of both results could be explained by the effect of factors such as topography, land use, soil type or biotic interactions [42].

In the case of bark beetles and ambrosia beetles, the female is responsible for locating and colonizing a new host [44]. To establish and reproduce, she must overcome the tree defenses, possibly the reason why subcortical species choose weakened or dead hosts [45]. In this study, the main environmental factor related to the abundance of 12 species was the percentage of maximum relative humidity, which was negatively related to the abundance of nine species, possibly because the species wait for the optimal time to take flight in search for hosts, as in lower humidity the trees develop more stress and are more susceptible to colonization [46]. On the other hand, the abundance of the species that did not show a

significant correlation with climatic factors could be explained by the vigor of the hosts, as is the case of *Tomicus piniperda*, which showed the highest abundance in *Pinus sylvestris* L. with low vigor values [47]. There is also the possibility of being correlated with the type of forest management, as it has been demonstrated for *D. mexicanus* in the State of Michoacan, Mexico, where the highest number of insects captured was obtained in forests under silvicultural management, compared to conserved forests [48].

Knowing the influence of climatic factors on the flight activity of bark beetles and ambrosial beetles provides the basis for monitoring and control strategies for species with potential to become pest, however, it is necessary to explore the relationship of other factors such as soil, physiology and genetics of the hosts and their interaction with predators.

CONCLUSIONS

This study has identified the relationship between climatic factors and the abundance of bark beetles and ambrosia beetles in an oak-pine forest in the northeastern Sierra of the State of Puebla. The greatest increase in abundance was obtained during the months from August to December, a period that coincides with the decrease in the percentage of maximum relative humidity. Sampling for 20 months collected 68.2% of the diversity of bark beetles and ambrosia beetles in the study area. To increase the percentage of sampling effort, it is recommended to extend the collection time or the number of traps.

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Productive potential and typology of the bean agroecosystem in the Papaloapan basin, Mexico

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ABSTRACT

Objective: analyze the municipal level production potential using simulation methods and typification of the bean agroecosystem based on the edaphoclimatic, socioeconomic, and technological characteristics present in the basin.

Design/methodology/approach: the potential yield of beans was mapped, the SWAT model set a total of 8423 HRU'S within the basin surface. Based on this, three clusters were generated for high (1-65-2.75 t ha⁻¹), medium (0.87-1.64 t ha⁻¹), and low (0-01-0.86 t ha⁻¹) yield.

Results: regarding the variables used to analyze the typology of clusters of the bean agroecosystem, Natural Resources (RN) had a P=6.842e-08 value; Technological Development (DT) a P=1.01e-06 value and Infrastructure Development (DI) a P=8.284e-06 value, while the Economic Development (DE) variable obtained a value of P=0.3564 and the Social Development variable (SD) a P=0.04867 value, therefore, in these last two, there were no significant differences among the three clusters.

According to the P values, the high cluster agroecosystem presents the ideal conditions to produce comapa black beans, while the medium cluster can be improved to optimize it. For its part, the low cluster must be addressed in the RN, DT, and DI variables to improve its potential and sustainability.

Findings/conclusions: high cluster areas, despite containing fewer municipalities, present a higher production biophysical potential (RN). The agroecosystem of the high and medium-yield clusters has a surface with edaphoclimatic, socioeconomic, and technological characteristics suitable for the productive development of the bean agroecosystem.

Keywords: agroecosystem, bean, SWAT model, typology, Papaloapan.

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INTRODUCTION

In Mexico, beans rank second in importance in the national agri-food sector, due to their socioeconomic importance, the extension of land for its cultivation, and *per capita* consumption (San German-Jarquín, 2010).

Beans are produced in two seasons: spring-summer and autumn-winter. Due to their high availability, low cost, and cultural tradition, at a national and regional level, about 70 varieties of beans distributed in seven groups are cultivated: black, yellow, white, purple, bay, pinto, and speckled (Lara, 2015).

The demand for black beans (*Phaseolus vulgaris* L.) concentrates in the central and southern areas of the country, with consumption close to 300,000 t per year. The main black beans-producing states in the humid tropical region are Chiapas and Veracruz states, in the dry tropical region Nayarit and Sinaloa states, and in the semi-arid highlands, Zacatecas, Durango, and Guanajuato states (Acosta-Gallegos *et al.*, 2000; López -Salinas *et al.*, 2011).

In Veracruz state, mainly black and opaque grain varieties are cultivated. In 2010, the Bean Program of the National Institute of Forestry, Agriculture and Livestock Research (INIFAP), through the Cotaxtla Experimental Field, registered the improved Comapa black variety, whose grains are opaque black in color and small, characteristics demanded by the local producers and consumers (López-Salinas *et al.*, 2011b). This variety has high yield potential, disease tolerance, and a high adaptation rate to tropical agro-environmental conditions.

In terms of biodiversity, culture, and water resources the Papaloapan Basin is a rich area. In this region, the land use is mainly for agricultural activities, representing approximately 85%. Due to its location and topography, the Papaloapan basin has different climates: 47% of the area is extremely hot, with average temperatures of 25 °C, 47% is temperate and 6% corresponds to cold conditions (Moreno *et al.*, 2003).

According to the diversity of bean grains the Papaloapan basin reports and the crop's importance, this research objective was to analyze the production potential, at the municipal level, using simulation methods and typification of the bean agroecosystem based on the edaphoclimatic, socioeconomic, and technological functions present in the basin.

MATERIALS AND METHODS

Study area

The Papaloapan basin is located on the southern slope of the Gulf of Mexico; its surface covers territories of the states of Puebla, Oaxaca, and Veracruz, totaling an area of 46,263 km² that represents 2.36% of the Mexican territory (Murillo and López, 2005).

Sub-basins Delineation

The SWAT software (Neitsch *et al.*, 2005) works at the basin and sub-basin levels. The model subdivides the basin into sub-basins, based on the topography of the study area (Narasimhan *et al.*, 2005; Garg *et al.*, 2011; Du *et al.*, 2006; Akhavan *et al.*, 2010; Guzmán *et al.*, 2004). For the present work, the total area of the Papaloapan Basin was taken. Using a Digital Elevation Model (DEM) with a 90×90 pixel acquired from (INEGI) we proceeded to generate sub-basins. At the end of the process, 168 sub-basins were generated for the entire study area.

Generation of Hydrological Response Units (HRU)

Once the sub-basins were created, the model subdivided them into hydrologic response units (HRUs) based on a single soil type, land use, and slope range (Narasimhan *et al.*, 2005; Garg *et al.*, 2011; Du *et al.*, 2006; Akhavan *et al.*, 2010; Guzmán *et al.*, 2004). For the HRU generation within the surface of the basin, a vector format series III soil map from the INEGI scale (1:250,000) was used. This database came from the spatial distribution of 4,418 soil profiles classified WRB (Paz-Pellat, 2018). The slope ranges were divided into 5 categories (0-3, 3-8, 8-15, 15-30, and >30%). By the end of the process, the model generated 8,423 HRU'S.

Generation and Assignment of Climate and agronomic management

For the introduction of the climatic data required by the model, the historical data of 1074 climatic stations of the national meteorological service was used, and the EPIC climatic generator (Sharply and Williams, 1990) produced the climatic statistics required by the model for each station.

Table 1 shows the considered management in the simulation of the Comapa Black beans variety within the total basin area.

Development of the municipal typology of the bean agroecosystem

The analyzed variables for the entire study area were: 1. Economic Development (DE); 2. Technological development (DT); 3. Social development (DS); 4 Infrastructure Development (DI); and 5. Natural resources (RN), following the synthetic statistics methodology by Uresti *et al.* (2016).

RESULTS AND DISCUSSION

The analysis of variance (ANOVA - R[®]) of the data generated by the SWAT model for the 2016-2020 period, indicates that there is a significant difference ($F_{1, 42110}=197.25$, $p=2.2e-16$) among the simulated years. By fitting the data model generated in R studio (R core team, 2021) for the five years (command: `negrocomapa <- lm(YLDth~Year, data = csvsimne9)`) and the emmeans function (CI=95) an average of the data was generated. The spatially explicit results are shown in Figure 1.

Based on the results of the simulated yields map in Figure 1 and the division of ranges by breaking natural breaks method (Lee *et al.*, 2019), the municipalities (280 municipalities) of the Papaloapan Basin were divided into three clusters (High: 1.65-2.75 t ha⁻¹; Medium: 0.87-1.65 t ha⁻¹; Low: 0.01-0.86 t ha⁻¹). Table 2 shows field data and simulated average yield at the municipal level for different regions of the basin.

Through a correlation analysis of the data assessed in the field, and those simulated by the SWAT model under the Pearson method in the R[®] studio, a value of $P=0.05$, $IC_{95} = -0.026$ to 0.99, and a weighted correlation coefficient of 0.9, which indicates that the model simulated with notable precision.

Table 1. Bean management introduced to the model.

Activity	Year	Operation	Input	Date
Soil Preparation	1	Cleaning	Rotary Hoe	September 1 st
	1	Fallow	Disk Plow Ge 23 ft	September 15 th
	1	Tillage	Tandem Disk Plw 14-18 ft	September 29 th
	1	Tillage	Tandem Disk Plw 14-18 ft	September 30 th
Crop establishment	1	Planting	COMAPA BLACK BEANS	October 1 st
Fertilization	1	1 st Fertilization	23-00-00 NPK (Kg ha ⁻¹)	October 1 st
	1	2 nd Fertilization	18-46-00 NPK (Kg ha ⁻¹)	October 1 st
	1	1 st Cultivator	Row cultivator	October 20 th
Cultivator	1	1 st Furrower	Furrow diker	November 5 th
Harvest	1		Harvest and kill operation	December 9 th

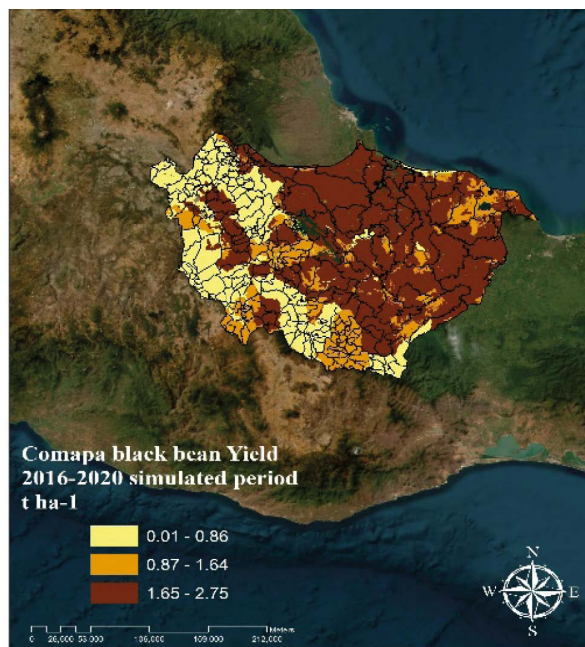


Figure 1. Simulation of the productive potential of Comapa black beans with the SWAT model in the Papaloapan Basin.

According to Figure 2, the analysis of variance of the RN variable showed a significant difference ($F_{1, 537}=17.01$; $P=6.842e-08$) in the three clusters.

In Figure 3, the analysis of the DE variable did not show a significant difference ($F_{1, 537}=1.03$; $P=0.3564$). For its part, Figure 4 shows that the DS variable showed a significant difference between clusters ($F_{1, 537}=3.0397$; $P=0.04867$).

The analysis of the variance of the DT variable showed a significant difference ($F_{1, 537}=14.167$; $P=1.01e-06$) in the three clusters. The behavior of the variable is shown in Figure 5. Finally, the DI variable showed values of $F_{1, 537} = 11.96$, and $P = 8.284e-06$, for which there was a significant difference between clusters. Figure 6 shows their analysis of variance.

Table 2. Yield of the Comapa black beans variety in municipalities of the Papaloapan Basin.

Municipalities of the Papaloapan basin	Cycle / year	Municipal average yield (kg ha^{-1}) field trial	average municipal yield (kg ha^{-1}) SWAT simulation	Field trial references
Isla, Veracruz	O-W 2009-10	2000	2200	López Salinas <i>et al.</i> , 2010
San Andrés Tuxtla, Ver.	O-W 2009-10	1649	1810	López Salinas <i>et al.</i> , 2012
	O-W/2011-12			López Salinas <i>et al.</i> , 2015
	O-W/2012-13			Tosquy Valle <i>et al.</i> , 2020
Acazacucan, Ver.	O-W/2011-12	1567	1810	Tosquy Valle <i>et al.</i> , 2020
Orizaba, Ver.	O-W/2011-12	1272	1255	Tosquy Valle <i>et al.</i> , 2020
Medellín, Ver.	O-W 2008-09	1292	1727	López Salinas <i>et al.</i> , 2010

O-I: Autumn-winter.

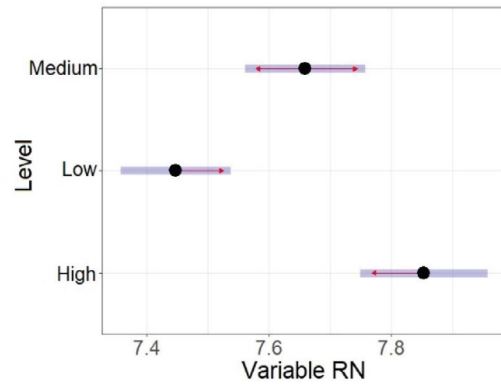


Figure 2. Behavior of the RN variable at the three cluster levels.

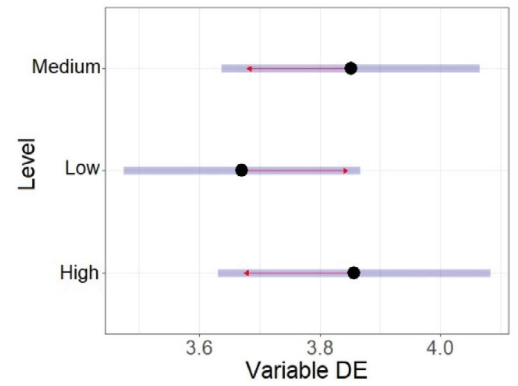


Figure 3. Behavior of the DE variable at the three cluster levels.

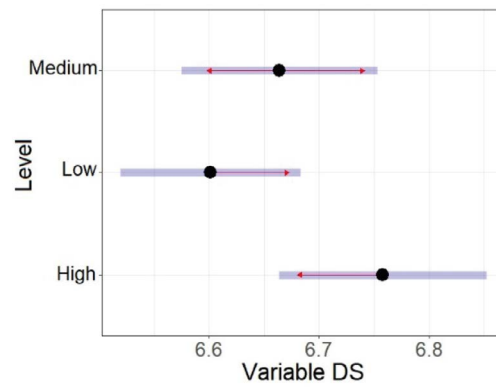


Figure 4. Behavior of the DS variable at the three cluster levels.

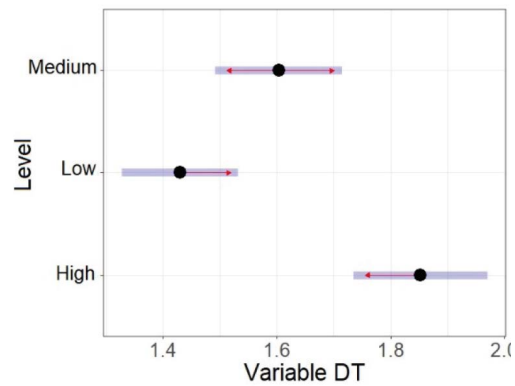


Figure 5. Behavior of the DT variable at the three cluster levels.

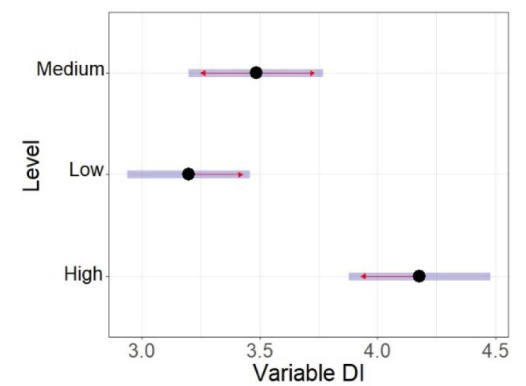


Figure 6. Behavior of the DI variable at the three cluster levels.

CONCLUSIONS

The main differences between clusters lie in the DT and DI variables indicators since the DE and DS variables show no significant differences. The high and medium-yield clusters have an ideal suitability for the productive development of the bean crop. Regarding the level that makes up the low cluster, it is important to attend to the context of the indicators that the variables RN, DT, and DI entail.

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Varietal description of two genotypes of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.)

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ABSTRACT

Objective: The objective of this research study was to obtain the varietal description of two varieties of manzano chili pepper in Las Montañas region, in central Veracruz, Mexico.

Design/methodology/approach: The varietal characterization module was established under greenhouse conditions. The markers recorded were in accordance with the International Plant Genetic Resources Institute for *Capsicum* and the Graphic Handbook for Variety Description of manzano chili pepper. The plants were characterized from seedling in greenhouse to adult plant. The agronomic management of the crop was carried out in accordance with the manual for the production of manzano chili pepper in Las Montañas, state of Veracruz.

Results: All qualitative markers were constant for the two varieties, MEXUVNE1-15-C2 and MEXUVCU1-16-C2, from seedling to fruit setting. In contrast, there were dissimilarities in plant height, and stem, leaf, flower, fruit and seed dimensions.

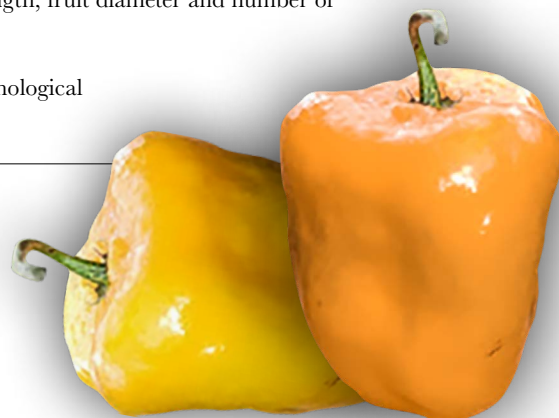
Study limitations/implications: The pandemic caused by COVID-19 was the main limitation, resulting in some markers not being recorded in a timely manner as indicated in the Graphic Handbook.

Findings/conclusions: Both varieties are very similar; however, the greatest distinction was in the quantitative markers, such as: plant height, fruit length, fruit diameter and number of seeds.

Keywords: High Mountains, novel variety, morphological markers, characterization.

INTRODUCTION

Capsicum is an economically important genus within the Solanaceae family (Hernández-Pérez *et al.*, 2020), it is made up by at least 31 species, although only five have



been domesticated: *C. baccatum*, *C. chinense*, *C. frutescens*, *C. annuum* and *C. pubescens* (Pérez-Castañeda *et al.*, 2015). The latter is the fifth most important species cultivated from the genus and has its origin in the high parts of the Andes; its introduction to Mexico was made at the beginning of the 20th century where it is cultivated in transition areas and of temperate climate, ranging from 1300 to 2400 masl (Pérez-Grajales *et al.*, 2004). Recent studies report the presence of this species in several states of the Mexican Republic such as Tamaulipas, Nuevo León, Chihuahua, Estado de México, Puebla, Oaxaca, Chiapas, Michoacán, Hidalgo and Veracruz (Hernández-Pérez *et al.*, 2020; Serna *et al.*, 2020).

Studies carried out by Leyva *et al.* (2018) evidence reservoirs of genetic material from *C. pubescens* with outstanding morpho-agronomic characteristics in family gardens and as associate crop with species of fruit and forest trees. However, there are still few studies that address aspects of identification and exploitation of the genetic material, morphological characterization of plant and fruit, and release of outstanding materials by producers for their use. In this sense, the production of manzano chili pepper in the region of Las Montañas, Veracruz, has not been outstanding because its cultivation is conducted primarily under rainfed conditions where the yield, use of improved varieties, and level of modernization are low, impacting directly on the productivity and profitability of the crop. Therefore, studies are necessary that lead to the varietal identification and characterization to increase the productivity of local farmers. Because of this, the objective of this research study was the varietal characterization of two genotypes of manzano chili pepper in Las Montañas, in the central zone of Veracruz, with registry purposes.

MATERIALS AND METHODS

The establishment of the varietal characterization plot was carried out in the Centro de Bachillerato Tecnológico Agropecuario No. 99 (CBTA 99) in the municipality of Coscomatepec de Bravo, located geographically at 19° 06' 58" LN, - 97° 02' 29" LW and altitude of 1396 masl. The experimental varieties MEXUVCU1-16-C2 and MEXUVNE1-15-C2, which come from manzano chili pepper fruits selected from plants with outstanding morphological characteristics, were characterized through the mass selection plan (Merino, 2019).

From each variety, 80 seeds that were free of pathogens were selected and then sown in black polyethylene 26×15 cm bags, peat moss[®] was used as substrate. Two seeds per cavity were deposited with the purpose of ensuring germination. The seedlings remained in the bags until they developed 8 to 10 true leaves. The plot was prepared before the transplant, to achieve a good sowing bed. During the transplant, a plant was deposited per cavity in the soil, at a depth of 0.4 m, distance between plants of 0.8 m and distance between rows of 1.5 m, to obtain a population density of 27,777 plants ha⁻¹. Because the soil is not homogeneous, the varieties were established under a completely randomized experimental blocks design with two replications. Each observation unit was constituted by one plant, with a total of 20 plants per block. The agronomic management of the crop was conducted in accordance with the manual for the production of manzano chili pepper for Las Montañas region (Andrés-Meza *et al.*, 2019).

Thirty-nine (39) varietal descriptions were recorded corresponding to each phenological stage according to the International Plant Genetic Resources Institute (IPGRI, 1995) for *Capsicum* and the Graphic Handbook for Variety Description of manzano chili pepper (SNICS, 2017). Descriptive statistics were used for the quantitative markers, such as the arithmetic mean, standard deviation, standard error, coefficient of variation and variance, which allowed estimating and describing the behavior of the different accessions in relation to each marker. For the qualitative markers, absolute frequencies were obtained for each category. All the analyses were conducted with the SAS/STAT[®] version 9.0. software (Castillo, 2007).

RESULTS AND DISCUSSION

Seedling

Both genotypes presented a medium pigmentation of the hypocotyl with green coloring of the oval type leaf and presence of anthocyanins in the stem. Seedlings present purple coloring of the hypocotyl, as well as intermediate pubescence (Figure 1). At 73 days after sowing (das), variety MEXUVNE1-15-C2 presented a seedling height of 11.02 ± 2.74 cm, and MEXUVCU1-16-C2 of 11.58 ± 4.19 cm (Table 1).

Mature plant

After transplant and during the growth period (194 das), nine qualitative markers were constant for both genotypes. It is important to highlight the presence of pubescence on the

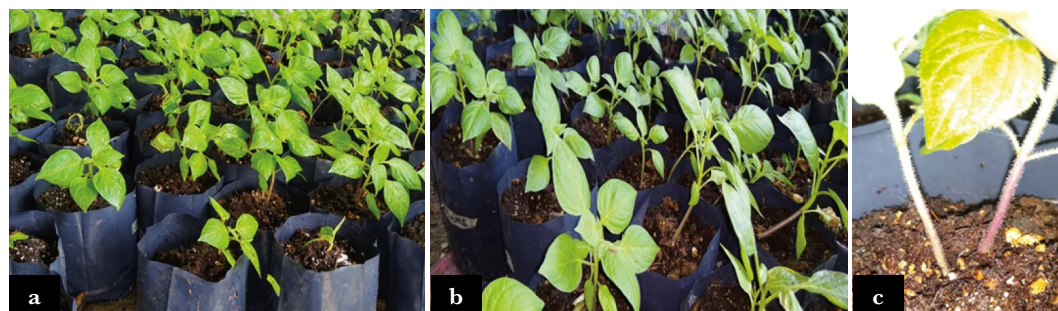


Figure 1. Seedling of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.); a) variety MEXUVCU1-15-C2, b) variety MEXUVCU1-16-C2, c) pubescence of the hypocotyl.

Table 1. Markers that correspond to a seedling in two varieties of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.).

Descriptor	Characteristics MEXUVNE1-15-C2	Characteristics MEXUVCU1-16-C2
Seedling: anthocyanin coloration of hypocotyl	Present	Present
Seedling height (cm)	11.02 ± 2.74	11.58 ± 4.19
Intensity of the anthocyanin coloration of the hypocotyl	Medium	Medium
Hypocotyl staining	Purple	Purple
Hypocotyl pubescence	Intermediate	Intermediate
Cotyledon leaf color	Green	Green
Cotyledon leaf shape	Ovate	Ovate

stem, elliptical shape of the leaf, dark color of the leaf, and absence of roughness on the leaves (Figure 2). When it comes to the quantitative markers, the genotype MEXUVNE1-15-C2 presented a plant height of 17.52 ± 6.50 cm, the number of shortened internodes from the first bifurcation was 0.84 ± 0.87 , the length of the internode of the lateral exterior branches was 3.45 ± 1.36 cm, the size of the lamina reached 9.92 ± 3.24 cm of length and 4.93 ± 1.72 cm of width, respectively. The genotype MEXUVCU1-16C2 presented a plant height of 16.53 ± 5.29 cm, the number of shortened nodes after the first bifurcation was 0.97 ± 0.90 ; likewise, the length of the internodes of the branches was 3.45 ± 1.36 cm and a lamina size of 10.91 ± 2.15 cm of length and 5.13 ± 1.17 cm of width (Table 2).

Table 2. Markers that correspond to the plant, stem and leaf, in two varieties of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.).

Descriptor	Características MEXUVNE1-15-C2	Características MEXUVCU1-16-C2
Plant: anthocyanin coloration of nodes	Present	Present
Plant: height (cm)	17.52 ± 6.50	16.53 ± 5.29
Plant: shortened internodes (after the first bifurcation)	Present	Present
Plant: number of internodes after the first bifurcation	0.84 ± 0.87	0.97 ± 0.90
Plant: length of internode on primary side shoots (cm)	3.45 ± 1.36	3.65 ± 1.32
Stem: intensity of the anthocyanin coloration of the node	Strong	Strong
Stem: pubescence	Medium	Medium
Petiole: attitude	Semi-drooping	Semi-drooping
Leaf blade: length (cm)	9.92 ± 3.24	10.91 ± 2.15
Leaf Blade: width (cm)	4.93 ± 1.72	5.13 ± 1.17
Leaf: shape	Elliptic	Elliptic
Leaf: intensity of green color	Dark	Dark
Leaf: profile of cross section	Concave	Concave
Leaf: rugosity	Absent	Absent

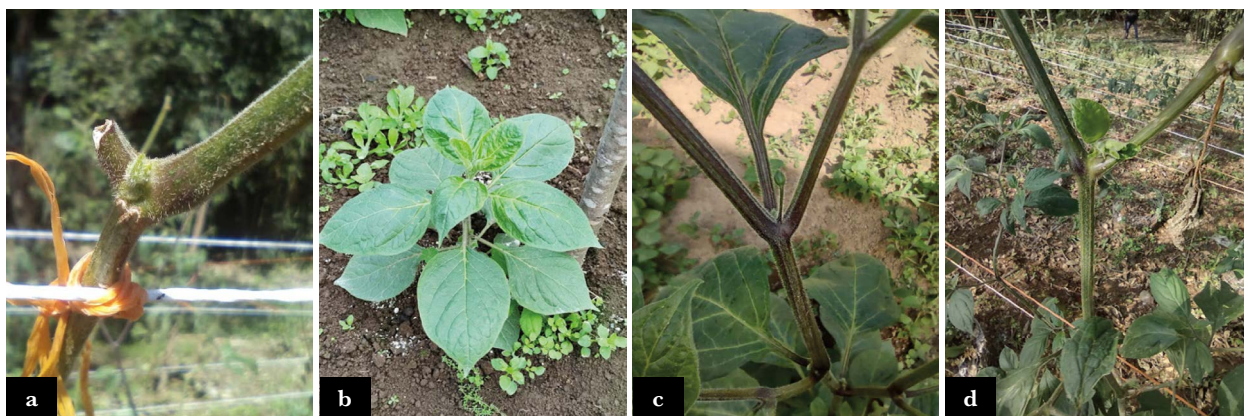


Figure 2. a) Pubescence of the stem, b) shape of the leaf, color, and absence of roughness, c) anthocyanin pigmentation of the nodes, and d) presence of shortened internodes after the first bifurcation.

Flowering

During the flowering period (213 das), the variety MEXUVNE1-15-C2 presented a plant height of 45.75 ± 19.10 cm, while the variety MEXUVCU1-16-C2 reached a height of 51.13 ± 18.76 cm (Table 3). The flower in both varieties has presence of anthocyanins on the anther, strong anthocyanin intensity of the petals, strong intensity of the style, and an upright stalk (Figure 3); likewise, the type of ovary that both genotypes present is superior.

Table 3. Characteristics of the flower in two varieties of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.).

Descriptors	Characteristics MEXUVNE1-15-C2	Characteristics MEXUVCU1-16-C2
Flowering plant height (cm)	45.75 ± 19.10	51.13 ± 18.76
Flower: anthocyanin coloration in anther	Present	Present
Flower: intensity of the anthocyanin pigmentation of petals	Strong	Strong
Flower: intensity of anthocyanin coloration in style	Strong	Strong
Peduncle: attitude	Erect	Erect



Figure 3. a) Petals: intensity of pigmentation, b) pigmentation of the anther, c) style: intensity of the pigmentation, d) growth habit of the stalk.

Fruit and seed

According to Pérez-Grajales *et al.* (2004) and Martínez-Estrada (2016), the manzano chili pepper fruit is a berry and can adopt different shapes depending on the number of locules. The variety MEXUVNE1-15-C2 presents a square shape with 2.70 ± 0.64 locules (Figure 4a and 4b), as well as fruit length and diameter of 4.78 ± 0.69 and 4.75 ± 0.72 cm, respectively. Likewise, it has a fruit weight of 41.96 ± 10.66 g and a pericarp thickness of 4.19 ± 0.80 mm (Figure 4c).

The variety MEXUVCU1-16-C2 also has a square fruit shape with 2.67 ± 0.66 locules (Figure 5a); it has a fruit length and diameter of 4.71 ± 0.69 and 4.57 ± 0.78 cm,

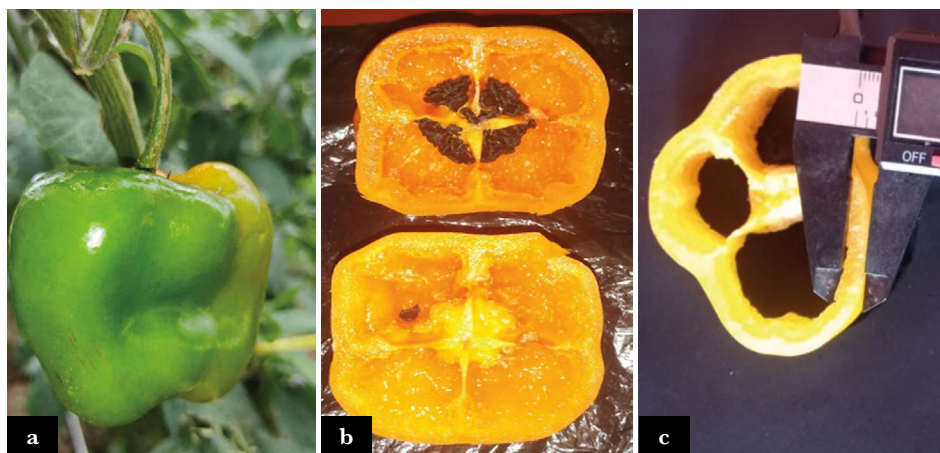


Figure 4. a) Shape of the fruit of variety MEXUVNE1-15-C2, b) number of locules, c) pericarp thickness.

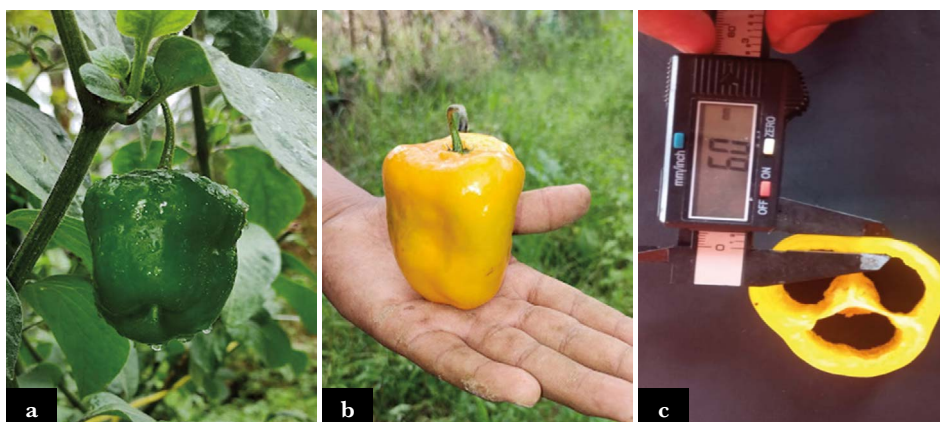


Figure 5. a) Fruit shape of the variety MEXUVCUI-16-C2, b) number of locules, c) pericarp thickness.

respectively. It has a dark green color before commercial maturity, while in maturity it becomes yellow with a dark and intense tonality (Figure 5a and 5b). The average weight per fruit was 38.93 ± 13.52 g, a pericarp thickness of 4.19 ± 0.81 mm (Figure 5c). According to Espinosa *et al.* (2014) and Escalera-Ordaz *et al.* (2019), the manzano chili pepper fruits that are cultivated under greenhouse conditions present superior or outstanding characteristics in comparison to the fruits cultivated in the open field and rainfed conditions.

Seeds of the manzano chili pepper are of medium size with dark brown color and hard testa, which makes its germination period longer than the other types of chili peppers within the genus *Capsicum* (Leyva *et al.*, 2018). The number of seeds per fruit for variety MEXUVNE1-15-C2 was 56.30 ± 18.50 , while for variety MEXUVNE1-16-C2 it was 51.22 ± 23.18 (Table 4).

Table 4. Markers for fruit in two varieties of manzano chili pepper (*Capsicum pubescens* Ruiz & Pav.).

Descriptors	Characteristics MEXUVNE1-15-C2	Characteristics MEXUVNE1-16-C2
Fruit: ratio length/diameter	Small	Small
Fruit: glossiness	Strong	Strong
Fruit: intensity of the green color at the beginning of commercial maturity	Dark	Dark
Fruit: intensity of the yellow color	Dark	Dark
Fruit: shape	Square	Square
Fruit: length (cm)	4.78±0.69	4.71±0.69
Fruit: diameter (cm)	4.75±0.72	4.57±0.58
Fruit: shape in cross section (at level of placenta)	Angular	Angular
Fruit: number of locules	2.70±0.64	2.67±0.66
Fruit: number of seeds	56.30±18.50	51.22±23.18
Fruit: thickness of pericarp (mm)	4.56±0.67	4.20±0.80
Peduncle: length (cm)	3.89±0.66	3.96±0.60
Peduncle: diameter (cm)	1.76±0.35	1.67±0.33
Fruit: depth of stalk cavity	Very deep	Very deep
Fruit: apex depth	Very deep	Very deep
Calyx: margin	Entire	Entire

CONCLUSIONS

There are similarities between the varieties MEXUVNE1-15-C2 and MEXUVCU1-16-C2 for the markers evaluated in each stage of seedling, adult plant and flowering. However, in the fruit setting stage they showed greater variation. These findings will allow starting the registry in the National Service of Seed Inspection and Certification (*Servicio Nacional de Inspección y Certificación de Semillas*, SNICS), on order to later produce seed and begin distributing it among producers.

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Family patio agroecosystem: role and empowerment of rural women in two communities

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ABSTRACT

Objective: To identify the role of women in the family patio agroecosystem and its relationship in the empowerment process, in two groups of women in the municipality of Medellín de Bravo, Veracruz, Mexico.

Design/methodology/approach: The research carried out is qualitative-quantitative, of a constructionist nature, and is based on the Research-Action-Participatory process, through adaptation of the management model for natural resource management in family farming.

Results: The psychometric results obtained in the Rosenberg Self-Esteem Scale indicate that the average response is 26.3, which indicates a normal self-esteem of women who work in the family patio agroecosystem, and positively impacts their human development, empowerment, and personal identity.

Limitations on study/implications: The work of rural women in the family patio agroecosystem is not recognized, so it is important that it be made visible, mainly to them, and that they be empowered in their being, through their actions.

Findings/conclusions: A total of 52 species were found, distributed among 15 fruit trees, 13 ornamental plants, 9 seasoning plants, 9 medicinal plants, 4 timber trees and only 2 vegetables. In the breeding of small species, 3 animal species are reported: 41 chickens, 2 pigs and 3 sheep.

Keywords: Family farming, visibility of work, rural women

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INTRODUCTION

In Mexico, the production units that are established in family agriculture are the plot, the family patio (backyard or orchard), and the school orchard. This type of agriculture is based essentially on family labor. The woman participates actively, mostly in the family patio, where she is the central axis, from the design, seed selection, sowing and farming tasks. Women decide the destination of production: auto-consumption, commercialization, or else exchange (barter). However, despite the importance of their work, it is not recognized,



even by them. This has made it impossible to visualize the psychosocial impact of this activity and process. Based on this, the role of women in the family patio agroecosystem was identified, as well as its relationship in the empowerment process in two groups of women in the municipality of Medellín de Bravo, Veracruz, Mexico.

MATERIALS AND METHODS

The study carried out was qualitative-quantitative, of constructionist nature (Schwandt, 2000), and based on the management model already mentioned (Álvarez *et al.*, 2011). Information gathering was made in two groups of women, one from the community of Rancho del Padre and the other in San Miguel, the two belonging to the municipality of Medellín de Bravo, Veracruz, Mexico. The field work was carried out from February to December, 2021. Field visits were conducted, and semi-structured interviews and psychometric tests about the participants' self-esteem were applied. In the events mentioned, work was done through knowledge exchange workshops, identifying the characteristics of each family patio, the tasks that are performed, the role that women play in the management, and its relationship with empowerment. Playful activities were carried out in the process, such as drawing their patio, identifying not only the components and the interactions between them, but also the role of women in the family patio's contribution to the diet, economy and recognition of women themselves as producers.

The phases in the management model adapted were motivation, diagnosis, community development and evaluation, in each phase of the process, to allow its feedback.

Phase 1. Motivation: visits were conducted to the family patios. The interested groups assume the commitment of participating and working in their patios, and that is the basis for the start of the model's application. **Phase 2. Diagnosis:** the stages that it includes are: delimitation of the study zones, according to the interest from groups and the conditions for community work, by performing documental diagnoses of the geophysical and socioeconomic conditions of the participating communities and of the municipality that they belong to (regionalization). Family patios were also identified, where each participant drew the composition of their family patio agroecosystem, on flipboard paper, and divided it into quadrants with the objective of understanding the importance of the location of the plants, and their name and use. Determination of the biodiversity of the plants cultivated and animals bred, and the destination of production, of family patios in the study groups. The methods used ranged from field visits and open interviews, to knowledge exchange workshops. **Phase 3. Community development:** a very important tool was the application of a semi-structured interview, with general data and questions about their perceptions and emotions when planting and harvesting, and about decision making in the family patio agroecosystem, and how this activity is related to their empowerment. One of the psychometric instruments most used to evaluate global self-esteem was Rosenberg's Self-Esteem Scale (EAR, Rosemberg, 1989), which is an instrument used to explore self-esteem and aspects such as personal auto-valuation and respect for herself. The level of self-esteem is important in relation to women's empowerment, in this case in relation to their work in the family patio agroecosystem.

RESULTS AND DISCUSSION

Motivation. It was the result from having visited the patios with the women, and it was the conformation of a group in the community of Rancho del Padre (six participants) and another in the community of San Miguel (three participants) in Medellín de Bravo, Veracruz.

Diagnosis. The delimitation of the study zones was in the municipality of Medellín de Bravo, Veracruz, which borders north with the municipalities of Veracruz, Boca del Río and Alvarado; east with the municipalities of Alvarado and Tlalixcoyan; south with the municipalities of Tlalixcoyan and Cotaxtla; west with the municipalities of Cotaxtla, Jamapa, Manlio Fabio Altamirano and Veracruz (INEGI, 2017). Different plant species are used mainly for auto-consumption, for food. The seasoning plants are cared for very carefully since they are useful to prepare foods and, in addition, for medicinal remedies; the fruit trees and for shade constitute a place for recreation and coexistence for families. The exchange (barter) of plants, seeds and fruits is common, and this practice contributes to the conservation of biodiversity and is an important element for self-recognition and wellbeing of women and their families. In the patios, 52 species were found: 15 fruit trees, nine medicinal, nine seasoning, two vegetables, 13 ornamental, and four woody. It was found that breeding of small animal species is carried out in four family patios. It is important to mention that 15 species present more than one use; 13 have two uses, and two have three uses. In the species with two uses, eight species stand out with medicinal use, six edible, six for seasoning, five ornamental, and one for timber/firewood. The species that presented three uses were basil (seasoning, medicinal and ornamental), and coconut palm (edible, woody and medicinal). These results show the knowledge there is in the use of their crops. The plants for seasoning are very well-cared for, since they are useful to prepare food and for medicinal use (Table 1).

The fruit trees and for shade constitute a place for recreation and coexistence. For the families, the exchange (barter) of plants, seeds and fruits is common. This practice contributes to the conservation of biodiversity and is an important element for self-recognition and welfare of women and their families. These results evidence the importance of the multifunctionality of the family patio, whose main contribution is the family's welfare. In the family patio agroecosystem, not only plant species were found, but also animal species, such as hens, sheep and pigs, which are the species that constitute animal breeding in the family patios of study.

Only four production units had animal breeding. The total number of animals was 46 (three sheep, two pigs, 41 hens). In general, they are located behind the house. The destination of this production is auto-consumption.

Community development. This phase was conducted through knowledge exchange workshops, and in them, the women recognized the importance of their work in the patios, making visible their duty and with it their being. Identifying the roles they carry out contributes to their empowerment and increases their self-esteem. To identify the importance of the location and management of crops in the family patios, the participants carried out a drawing of their patio where they showed the elements of the family patio agroecosystem that they manage. A piece of paper divided into six quadrants was used.

Table 1. Species with more than one use.

Scientific name	Part of the plant used	Used	Used forms	Used Numbers
<i>Agave</i>	Leaves Complete plant	Seasoning Ornamental	Food Ornament	2
<i>Bougainvillea</i> sp.	Flower	Medicinal Ornamental	Infusion to relieve cough Ornament	2
<i>Carica papaya</i>	Fruit Fruit and green leaves	Edible Tenderize	Fresh & onserved Tenderenze meat	2
<i>Citrus limon</i>	Fruit Leaves	Edible Medicinal	Beverage and food Infusion for relax	2
<i>Cocos nucifera</i>	Fruit Meat Water Leaves Trunk	Edible Medicinal Construction Construction	Food Beverage House roof Structure of a house	3
<i>Diospyros nigra</i>	Fruit	Edible	Fresh and dessert	1
<i>Dysphania ambrosioides</i> sp.	Leaves	Seasoning Medicinal	Food Infusion for deworm	2
<i>Mentha spicata</i>	Leaves	Seasoning Medicinal	Food Infusion for digestion	2
<i>Musa paradisiaca</i> sp.	Fruit Leaves	Edible Seasoning	Food Cooking tamales	2
<i>Persea americana</i>	Fruit Leave Seed	Edible Cosmetic Medicinal Cosmetic	Dip Hair conditioner Infusion, anti-inflamatori and pain control Darken eyebrows and hair	4
<i>Pinus</i>	Trunk	Woddy Construction	Firewood for cooking Structure of a house	2
<i>Salvia rosmarinus</i>	Leave	Seasoning Medicinal	Food Astringent in infusion	2
<i>Sansevieria trifascia</i>	Leaves	Medicinal Ornamental	Infusion, anti-inflamatori	2
<i>Tagetes lucida</i>	Flowers and leaves	Medicinal Ornamental	Infusion for relax Ornament	2

The participants drew the distribution of their crops and animal breeding, organizing the activities into a hierarchy according to the quadrant in which they drew them, as well as their importance and destination (Figure 1).

In the drawings, they described and counted the elements found. In the the participants' drawings analyzed, it was identified that ornamental plants are generally in front of the house or in the entryway, and they have a relationship with the beauty and emotional wellbeing they provide.

Animal breeding was placed behind the house, the fruit trees are located around the house without a specific location, the shade allows the house to be cooler and in the open spaces it provides places for recreation and relaxation, in addition to giving access to fresh fruit during the whole year.



Figure 1. Drawings of the family patio agroecosystem made by the participants.

The design of their patios has aspects that range from emotional wellbeing to satisfaction over having the availability of fresh foods, and the comfort of having their seasoning and medicinal plants accessible. The family patio agroecosystem generates positive emotions such as joy, satisfaction, effort and pleasure which impact their self-esteem directly and positively, by making their own decisions and deciding when and where to plant, which gives them assurance and self-confidence, valuing their being and their doing.

The rural women interviewed mention that this production unit helps the family economy by having fruits, and edible and medicinal herbs. They share that the fruits are healthier and more nutritious, which supports their diet. The care for the family patio agroecosystem has been a family and cultural inheritance that has been transmitted from generation to generation.

The Rosenberg Self-Esteem Scale (EAR, Rosenberg, 1989) is an instrument to explore self-esteem and aspects such as personal self-valuation and respect for oneself. The level of self-esteem is important in relation to women's empowerment, and in this case it is related to her work in the family patio agroecosystem. This scale includes 10 items in the content, half are stated positively and the other half negatively (Figure 2).

The average resulting score was 26.3, which indicates that the mean of self-esteem of rural women from the communities intervened is normal according to the indicators described before. Only one subject showed a higher self-esteem. The global results suggest that the work in the family patio agroecosystem impacts positively in the human development of the women, and particularly in their self-esteem (what a person feels for him/herself) with close relation with self-knowledge.

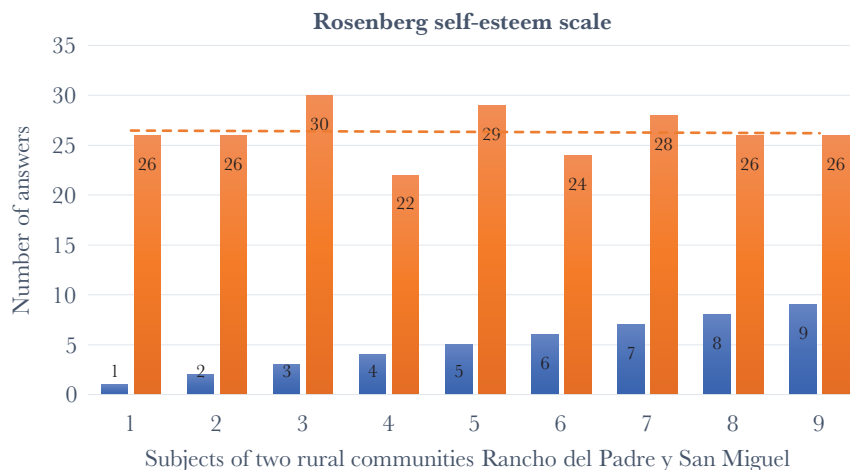


Figure 2. Graph of self-esteem results.

Authors such as Alcántara (2001) defines self-esteem as attitude, and it is the habitual way of thinking, loving, feeling and behaving with themselves, which is a very important factor in the identity of rural women in Medellín de Bravo. Self-esteem impacts their self-concept, which resides in the formation of their personality, since it has to do with social competence and influences how the person feels, thinks, learns and values him/herself, how they relate with others and, definitely, how they behave (Clemes and Bean, 1996; Clark, Clemes and Bean, 2000). When making decisions in the family patio agroecosystem, a positive impact is generated and this contributes to creating more and better opportunities for rural women of our country.

CONCLUSIONS

In Mexico, in the family patio agroecosystems, rural women are perceived to be in a space where they make their own decisions. The work by women in the family patio agroecosystem has served as a strategy to contribute to the diet thanks to the direct access to plants, fruits and breeding of small animal species. The women from the communities of Rancho del Padre and San Miguel are of the opinion that fruits from their patios are healthier, more nutritious, and that their ornamental plants provide them joy, satisfaction and pleasure. They have access to plants for cooking, medicinal plants, and impact their self-esteem directly and positively, contributing to create more and better opportunities for rural women. This study goes beyond a simple exploration, since the exchange of ideas makes it a place of learning.

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Use of Theory of Change (ToC) as a methodological approach to evaluate the papaya Maradol Regional Development Program in the Mixteca Poblana region

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ABSTRACT

Objective: To determine the extent to which the use of the Theory of Change (ToC) as a methodological approach is appropriate to assess the medium and long term effects generated by the Maradol Papaya Program in the Mixteca Poblana region (Puebla, Mexico).

Design/methodology/approximation: A case study was conducted with qualitative approach with a phenomenological approximation directed at the validation of the ToC as methodological tool for the assessment of programs, through Documental Research methods, observation and interviews with producers and key informants for the study.

Results: The design of the route or pathway of change allowed a punctual definition of the key variables for its operation and measurement as part of the assessment exercise. The use of qualitative methods (in-depth interviews) allowed an appropriate valuation of qualitative variables, such as learning, identification of quantitative variables (production volumes, production costs, etc.) that can be made with conventional methods (questionnaires) without this representing any methodological conflict.

Limitations on the study/implications: Since it is a case study, its generalization is not appropriate. However, there are elements that can be suggested as a general premise, such as the recommendation of the use of mixed methods in the assessment of programs and projects.

Findings/conclusions: The Theory of Change (ToC) is a valuable tool in identifying change variables through the design of the route or pathway change; however, this exercise has great validity and wealth if it is constructed in a participative way. The use of mixed methods (quantitative and qualitative) is suggested for this type of assessment.

Keywords: Theory of Change, program assessment, Maradol papaya.

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INTRODUCTION

In the Mixteca Poblana region (Puebla, Mexico), there is not a great diversification of crops, although the region has adequate soil and climate conditions for it. Facing this situation, strategies were established to change the existing social situation, one of them being the Maradol papaya Regional Development Program in the Mixteca Poblana.

Since August of 2002, Fundación Produce from Puebla has carried out an Integral Program for training, technical assistance, seedling production, fruit production, post-harvest management, and commercialization of Maradol papaya (*Carica papaya* L.) in the Mixteca Poblana region, supported by several institutions and taking advantage of the soil and climate conditions (Hernández, 2004; Jiménez, 2013). The proposed study contributes to the knowledge of the impact of this production model in the municipalities of the Mixteca Poblana where the Program was implemented, and seeks to determine the validity of the use of the Theory of Change as a methodological approach for this type of assessment. Based on this, the study determined the extent to which the ToC is useful to obtain data and to analyze results related with the Program's quantitative variables such as income, costs and others, in addition to defining the way in which the use of the ToC complements the conventional assessment approach, centered on the use of questionnaires with a mainly quantitative approach.

MATERIALS AND METHODS

A qualitative approach was used; the scope is the Mixteca Poblana region during the 2020-2021 period; the target population was Maradol papaya producers, men and women who are part of a Crop Development Program. The population of study was n=16 producers, the design used is phenomenological and case study. The variables were technological change, economic income, generation of family jobs, and decrease in temporary family migration. Given the qualitative nature of the study, the data were obtained through in-depth interviews, which were applied during the period of March to April, 2021. An interview guide was designed which was applied to producers to obtain information. The interviews were recorded and systematized through transcriptions that served for data analysis using the ATLAS.ti software. To implement the Theory of Change approach, a design of the pathway of change was made, as described in the next section, which was used to determine the variables and to explore deeply during the interviews.

RESULTS AND DISCUSSION

The fundamental basis of the assessment exercise from the approach of the Theory of Change is the construction (ideally in a participative way) of the so-called "route of change" or "pathway of change". This exercise consists in the proposal of a chain of results, going from the levels of products-effects-impact to complete the visualization of the change expected in terms of results or variables of change, which at the same time should be set out in a way that they can be measured in an assessment exercise. In this sense, the pathway of change represents the logical model of change expected, so that it allows the identification of variables of change that are relevant for their measurement in the assessment exercise (Weiss, 1995). Figure 1 shows the result of the construction exercise of the pathway of change for the Maradol papaya program in the Mixteca region. The products signaled that correspond to a first level of results were all given to the beneficiary population during the project's implementation period. The next level of results, corresponding to the effects, was developed through a certain period of time, and some were maintained until today as will be evident further ahead.

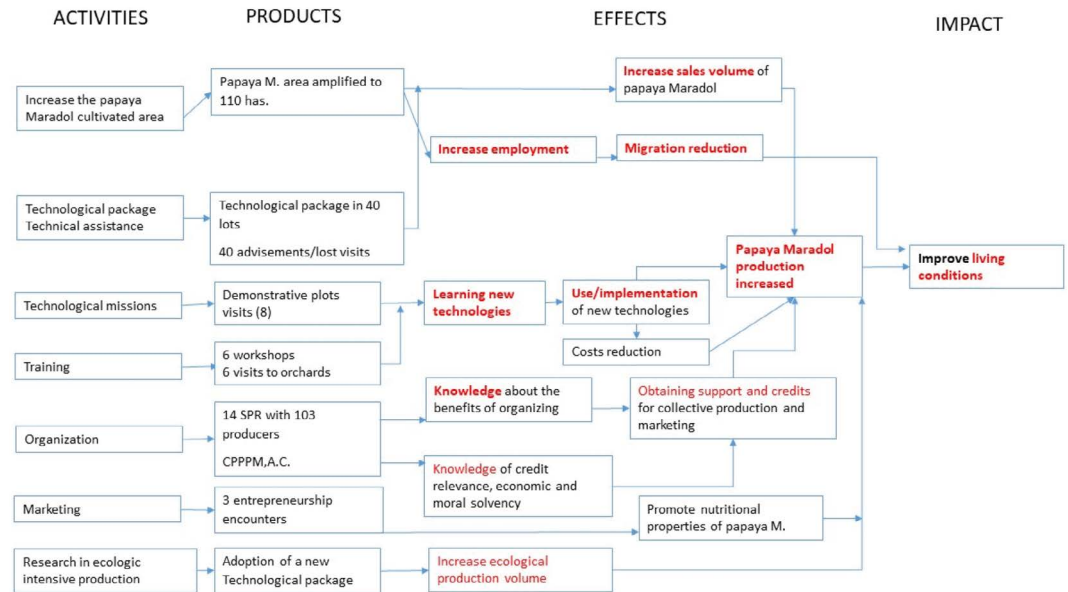


Figure 1. Logical model (route or pathway of change) of the Maradol papaya program in the Mixteca Poblana region. Source: Prepared by the authors.

Evidently, and from a systemic perspective, new effects and several elements of the context which have contributed to the expansion, or have limited these effects, emerged. These elements correspond to the changes derived from public policies, public administrations, the macroeconomic context, as well as from market flows, in addition to all the elements that are found outside the control of the program and actors. In order to respond to the question related to the relevance of the use of the ToC to assess the program, it is necessary to consider the nature of the variables to be measured. Variables such as “increase in production”, “volume of sales”, and “increase in levels of employment” are variables of a merely quantitative nature, so their measurement is relatively easy to record. However, variables such as “use/implementation of technologies”, “learning”, and “knowledge” are rather more complex, and therefore, their measurement corresponds to this complexity. To be able to conduct an exercise considering qualitative data collection, the study began by making a proposal of questions that allow the construction or operationalization of the variables in question, and thus to be able to value the relevance of the use of the ToC in assessment exercises.

Implementation of new technologies

The first variable to measure was the use/implementation of new technologies, which corresponded to the idea of “technology adoption” and which was addressed by addressing which components of the technological package were implemented, which were not, and why it was not done. Therefore, the relevant question is: Is there a relation between the main technological changes promoted by the Maradol Papaya Regional Development Program in the Mixteca Poblana region, in relation to the change of income, employment and migration between participating families from the Mixteca Poblana region, during the 2002-2008 period?

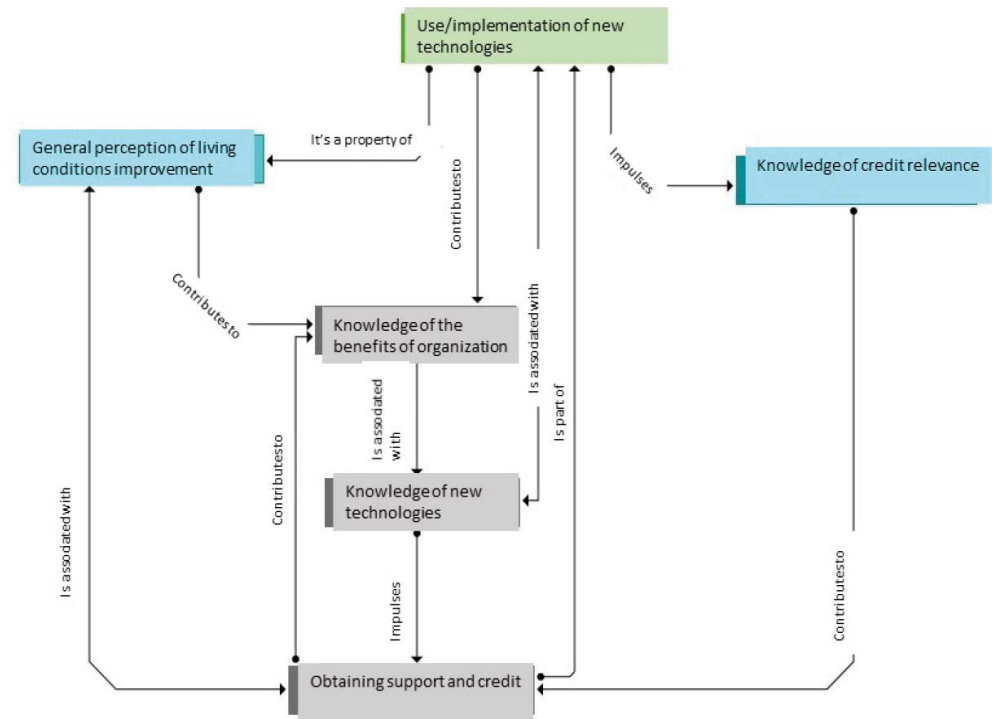


Figure 2. Semantic network for the use/implementation of technologies. Source: Prepared by the authors.

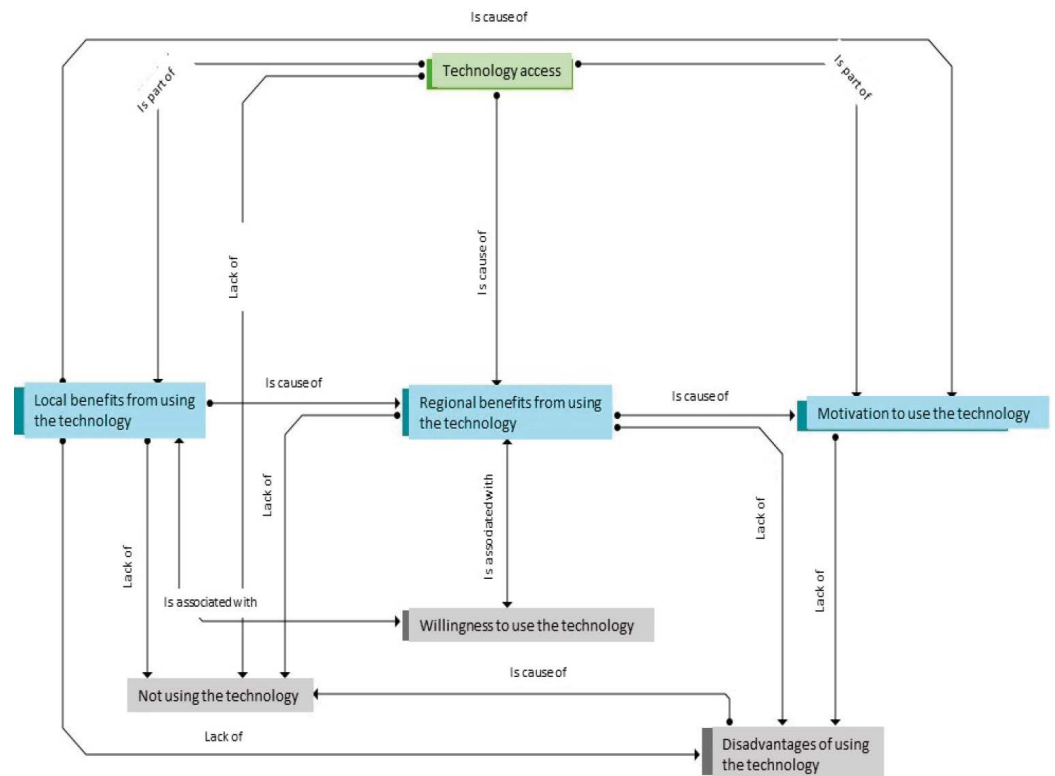


Figure 3. Semantic network for access to technologies. Source: Prepared by the authors.

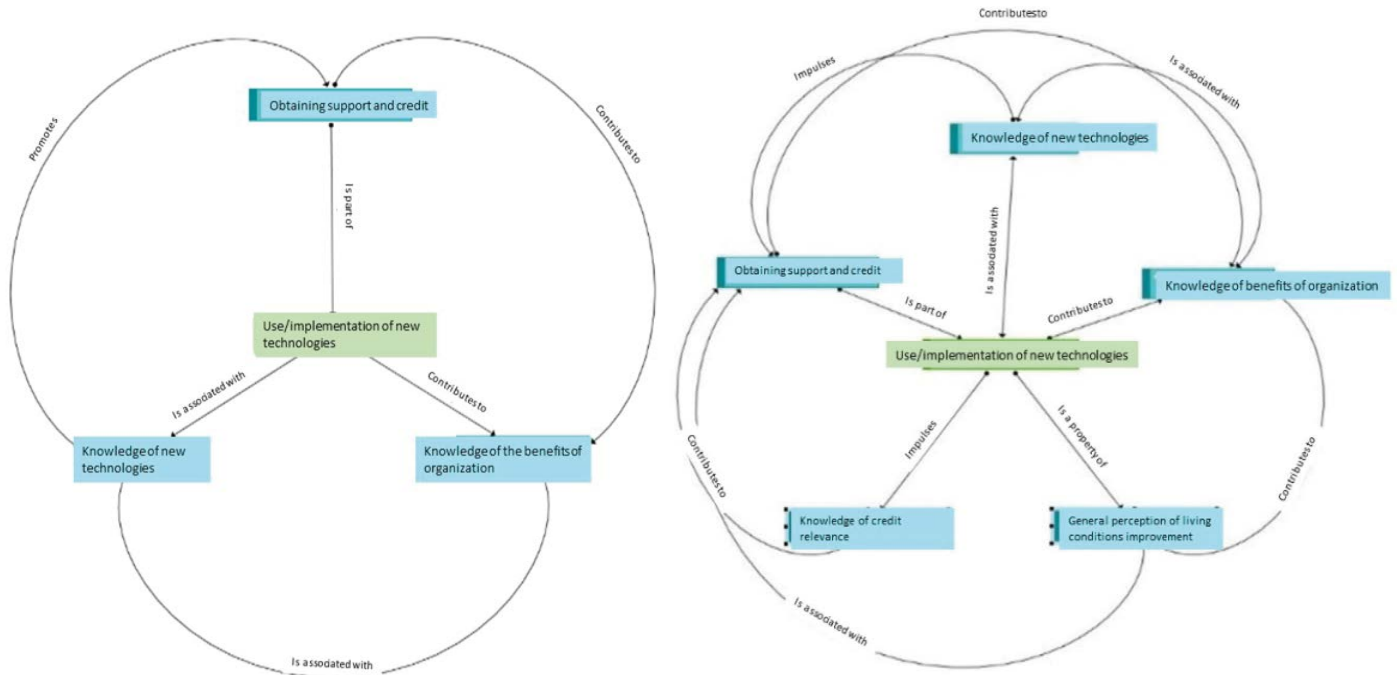


Figure 4. Semantic networks A and B for the use/implementation of technologies. Source: Prepared by the authors.

According to the analysis of semantic networks of the interviews performed, in the case of the use/implementation of new technologies, it is a property for the perception of improvement of living conditions and it promotes knowledge of the importance of credit, contributing to knowledge of the benefits of being organized and is associated to knowledge of new technologies. At the same time, the perception of the improvements of living conditions contributes to knowledge of the benefits of being organized. Knowledge of the importance of credit contributes to obtaining supports and subsidies and credit, and knowledge of the benefits of being organized is associated to knowledge of new technologies, which at the same time promotes obtaining supports and subsidies and credit (and is part of the use/implementation of new technologies).

Access to technology is part of the local benefits of use of technology and motivation to use said technology, and it is a cause for the regional benefits of its use. The local benefits contribute to regional benefits and they are cause for the motivation to use the technology, and this is associated with the willingness to retake the technology, while the lack of access has abandonment as consequence and causes disadvantages in the use of technology.

Technological changes

For this point, the following question was asked: Which are the main technological changes adopted by the family and the results obtained at the level of production with the Program?

NETWORK A. Use/implementation of new technologies: it is directly associated with knowledge of new technologies, and also with knowledge of the benefits of being organized which promotes obtaining supports and subsidies and credit, which in turn contributes to

knowledge of the benefits of being organized. Use/implementation of new technologies contributes to knowledge of the benefits of being organized that is associated to knowledge of new technologies.

NETWORK B. Use/implementation of new technologies drives the knowledge and importance of credit, contributing to obtaining supports and subsidies and credit, which promotes knowledge of new technologies. Use/implementation of new technologies contributes to knowledge of benefits of being organized which is associated to knowledge of new technologies. Use/implementation of new technologies is a property of the general perception of improvements in the living conditions that contribute to knowledge of the benefits of being organized, which contributes to obtaining supports and subsidies and credit. Finally, the main technological changes established (Figure 5) signal that the three main ones are the use of new varieties that are more resistant to virosis. Also, when the papaya tree has enough production, the plants with virus are eliminated. Finally, if a plant is sown, the buyer is not interested in the shape of the fruit, in addition to the seed having a considerable cost.

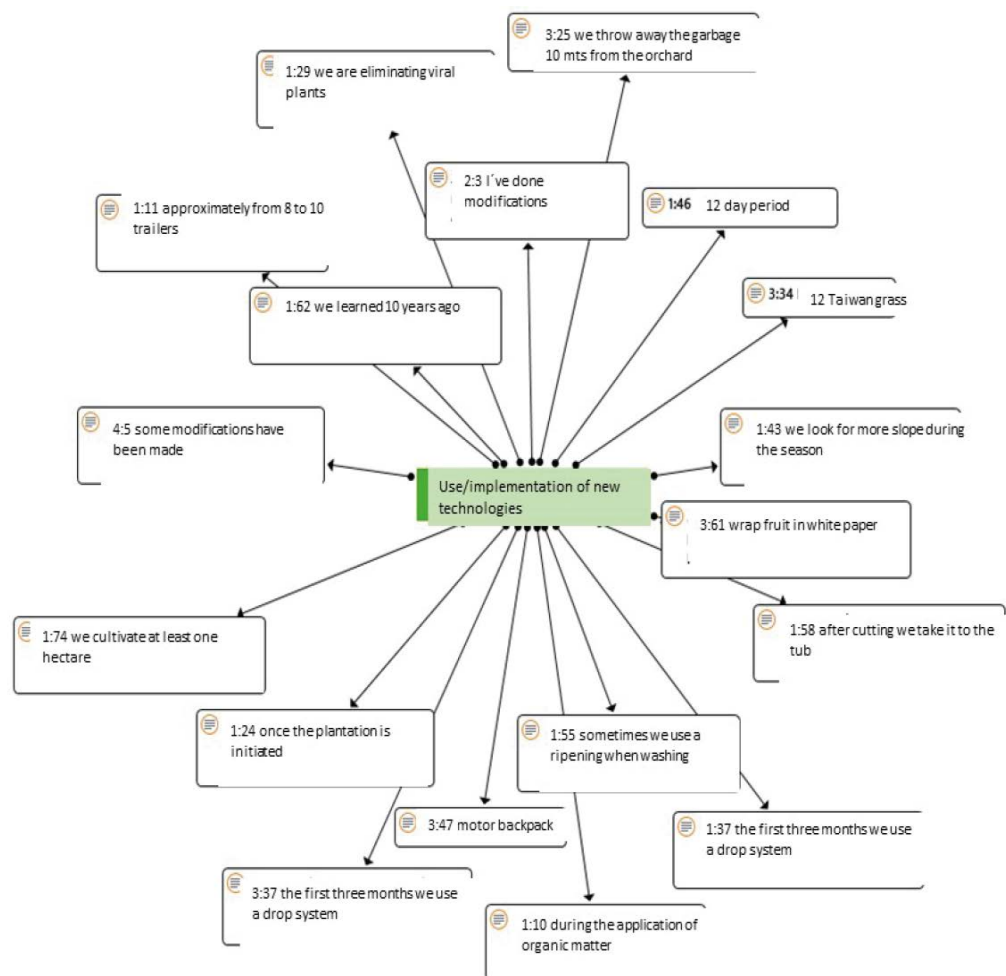


Figure 5. Main technological changes established. Source: Prepared by the authors.

In general, it is considered that the ToC is a valuable tool for the assessment of programs and projects such as the Maradol papaya Program in the Mixteca Poblana, if the following conditions are fulfilled:

Participation. The wealth of use of the ToC comes from the diversity of perspectives during the design stage of the pathway of change (Aspen Institute, 2004), as well as the identification of variables and actors (men and women) related to it, which is why with more diversity, inclusion and tolerance in the participation there will be greater wealth in the results. For the case of this exercise, the pathway of change was designed a posteriori as part of this study and was only validated with some key actors, which does not invalidate the exercise, but it does limit it to a unique perspective. Therefore, application of the ToC and its components is recommended, based on a mapping of actors that allows the identification of all the interested parties and their inclusion (Retalaza, 2010; Rogers, 2000).

Nature of the variables. Depending on the nature of the variables, the ToC has more or less relevance. In this sense, to identify and measure quantitative variables, it does not contribute more than any other assessment approach could. However, regarding precisely the dialogue process of the identification and selection of relevant variables, the ToC is more than appropriate, since it promotes a multiplicity of perspectives and their non-numerical valuation (qualitative), which is pertinent for variables such as learning, knowledge, and others.

Type of assessment. The ToC is relevant to assess results, particularly to assess effects, since the products are commonly valued by quantifying them while several effects are of a qualitative nature (Centre of Excellence for Evaluation, 2012).

CONCLUSIONS

The Theory of Change is a useful tool to assess the effects and the impact of the Program; there is a greater participation of local actors in the process of data obtention, and a relationship between the main technological changes with the Program with the use/implementation of new technologies.

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PCR molecular identification of the fall armyworm in the Chontalpa region, Tabasco, Mexico

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ABSTRACT

Objective: To perform the first molecular characterization of the barcoding COI region of the *S. frugiperda* found in Tabasco and to test whether it can reliably identify the strain detected in the said state.

Design/Methodology/Approach: We collected *S. frugiperda* specimens using four pheromone traps placed in corn plots. Subsequently, we implemented DNA extraction, PCR with LCO-L/HCO-L primers, and the sequencing of six individuals captured in the traps.

Results: After aligning the sequences, we developed a phylogenetic tree, determining that *S. frugiperda* belongs to the rice strain (RS).

Implications: Identifying the *S. frugiperda* strain is necessary for management purposes, since different strains may require different control methods.

Findings/Conclusions: Our study revealed that the isolates from Chontalpa, Tabasco, Mexico, match the RS fall armyworm reported in other latitudes. Consequently, this is the first report to identify this strain of *S. frugiperda* in Tabasco. Our findings are relevant because this species can potentially become a pest in sugarcane- and rice-growing areas of the state.

Keywords: *Spodoptera frugiperda*, Rice, Barcoding, Pests.

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INTRODUCTION

The genus *Spodoptera* (Guenée, 1852) (Lepidoptera: Noctuidae) comprises 30 species widely distributed in the Americas (Acosta, 2021). The genus includes four pest species of economically relevant Noctuidae that are highly polyphagous (EPPO, 2015). One of these is *Spodoptera frugiperda* (Smith, 1797), commonly called “fall armyworm” (FAW) (Casmuz *et al.*, 2010; Paredes-Sánchez *et al.*, 2021), an invasive pest species native to the Americas that has rapidly spread around the world. It has been found in Africa (2016), India (2018), China and Egypt (2019), Australia (2020), the Canary Islands in Spain, and Saudi Arabia (2021), where it has damaged corn crops (*Zea mays* L. (Poales: Poaceae)) (Paredes-Sánchez *et al.*, 2021; Varshney *et al.*, 2021).

Spodoptera frugiperda is a highly polyphagous pest that attacks more than 350 commercial and non-commercial hosts within 76 plant families (Maruthadurai and Ramesh, 2020; Montezano *et al.*, 2018) and causes significant economic damage to host crops such as rice (*Oryza sativa* L. (Poales: Poaceae)), cotton (*Gossypium* spp. (Malvales: Malvaceae)), sorghum (Poales: Poaceae), peanut (*Arachis hypogaea* L. (Fabales: Fabaceae), and grasses (Casmuz *et al.*, 2010). *Spodoptera frugiperda* has such an ample host range partly due to the presence of two species populations—called rice strain (RS) and corn strain (CS)—with varying host plant preferences; hence their designation as “host strains” (Pashley, 1986).

Since morphological techniques are not reliable during the larval stage of the pest, other identification tools are needed. Specific polymerase chain reaction (PCR) tests constitute a reliable method to correctly identify the FAW species from the moment they hatch and during the larval stages, based on the DNA of specimens found in a given region or state.

DNA-based methods therefore provide an independent and universal identification tool applicable to all stages of life (Mahat *et al.*, 2021). For this reason, identifying invasive species through DNA barcoding is increasingly recommended and performed globally (Floyd *et al.*, 2010; Phillips *et al.*, 2019; Yousaf *et al.*, 2022). During the barcoding process, a region of the mitochondrial cytochrome c oxidase subunit 1 (COI) gene is used to identify species of economic and ecological relevance. In the case of pests, this process contributes to the success of management programs based on the correct identification of the organism and its host (Yousaf *et al.*, 2022). This paper presents the sequencing of the proposed barcode region of the COI gene using adult individuals of *S. frugiperda* collected from corn plots. We use these COI gene data to 1) test whether the barcoding of the COI region can reliably identify *S. frugiperda* strains, and 2) report the first molecular characterization of *S. frugiperda* in the State of Tabasco.

MATERIALS AND METHODS

Insect collection

The adult *S. frugiperda* specimens were collected in corn fields of the Huimanguillo Experimental Field of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP-CEHUI) during 2021-2022. Pherocon Faw™ pheromone traps (Ferommis®) were used to capture adult males. All adult specimens were preserved in ethanol (95%) and stored at $-80\text{ }^{\circ}\text{C}$ in the phytopathology laboratory of the Colegio de Postgraduados–Campus Tabasco.

DNA extraction, amplification, and sequencing

The whole insect was used for the extraction of the DNA. Genomic DNA was extracted from adult insects according to protocol, using the cetyltrimethylammonium bromide (CTAB) insect DNA extraction method (Harrison *et al.*, 1996; Brown *et al.*, 2006). The DNA was resuspended in 30 μL of ultrapure water and stored at $4\text{ }^{\circ}\text{C}$. The barcoding COI region was amplified using the universal primer pair LCO1490-L (5'-GGTCWACWAATCATAAAGATATTGG-3') and HCO2198-L (5'-TAAACTTCWGGRTGWCCAAARAATCA-3')—which are slightly modified forms obtained by Nelson *et al.* (2007) from the primers designed by Folmer *et al.* (1994). Every 25 μL of the polymerase chain

reaction (PCR) mix contained 5X reaction buffer, 50mM MgCl₂, 100 ng of each primer, 100 μM each of dATP, dCTP, dGTP, and dTTP (Invitrogen, USA), 0.5 U of Mango TaqTM (Bioline, United Kingdom), and 50 ng of genomic DNA. PCR temperature cycles were performed in a C100 Thermal Cycler (Bio-Rad, Hercules, CA) as follows: initial denaturation cycle at 94 °C for 2 min, then 35 cycles at 94 °C for 30 s, followed by 50 °C for 45 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were visualized using electrophoresis on 2.0% agarose gel stained with ethidium bromide. Sequencing was performed in both directions with amplification primers using the 3500xl Genetic Analyzer (Applied Biosystems, USA) at the Instituto de Biotecnología of the Universidad Nacional Autónoma de México.

DNA sequence analysis

Sequences were refined and manually edited using the Bioedit 7.2.5 software (Hall, 1999). To secure their identification, several searches were conducted for each sequence in the Basic Local Alignment Search Tool (BLAST) system of the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov>). The ClustalX 2.1 software (with its default settings) was used for sequence alignment (Thompson *et al.*, 1997). Based on the *S. frugiperda* sequences, a phylogenetic tree was developed using the Molecular Evolutionary Genetics Analysis (MEGA) XI software (Tamura *et al.*, 2021). Data were subjected to maximum likelihood (ML) analyses based on the K-2 parameter model and evaluated for bootstrap analysis with 1,000 replicates, in order to estimate the stability of the inferred subclades. The outgroup for analysis was *Bombyx mori* L. All sequences in the present study (n=6) have been submitted to GenBank. In addition, reference sequences from GenBank (Table 1) were downloaded and incorporated into the phylogenetic analysis. To classify the strain in our phylogenetic analysis, we included barcodes designated as references for RS (Accession No. U72977) and CS (Accession No. U72974) in previous studies (Maruthadurai and Ramesh, 2020; Nelly *et al.*, 2021; Sarr *et al.*, 2021).

RESULTS AND DISCUSSION

Specimens

Forty-five adult males were collected from four traps located on the perimeter of corn plots between December 14, 2021, and March 15, 2022. Since the specimens sequenced in this study come from traps placed on the edges of corn plots, we do not have detailed data on the preferred host plants of *S. frugiperda*. However, larvae were indeed observed in corn plants at INIFAP-CEHUI.

Molecular identification and analysis

The *S. frugiperda* barcoding region COI was easy to amplify and sequence. According to other researches, sequences were easily aligned easily, given the absence of insertions and deletions (Hebert *et al.*, 2003). The mitochondrial DNA cytochrome c oxidase subunit 1 (COI) gene is commonly used to identify biotypes and study insect genetics (Kasambala Donga and Meadow, 2018). Cock *et al.* (2017), Maruthadurai and Ramesh (2020), and Nelly *et al.* (2021) recently observed that the amplifications of this barcoding region for

the molecular characterization of *S. frugiperda* produce a single, thick, and clear band with amplicons of ± 700 bp.

The six samples selected for barcode analysis generated a visible PCR product of the expected size (approx. 680 bp) representing *S. frugiperda*. These PCR products generated usable DNA sequences. All six sequences in this study were submitted to the GenBank databases on March 12, 2022. The sequences were identified as *S. frugiperda*, after nBLAST analyses of the data of these COI sequences were carried out in the NCBI GenBank database. Our findings are supported by a 100% similarity in sequence and coverage data. The sequences were trimmed to 643 bp and used in the phylogenetic analysis (Figure 1). For comparison purposes, 26 GenBank sequences were downloaded, including *S. frugiperda*

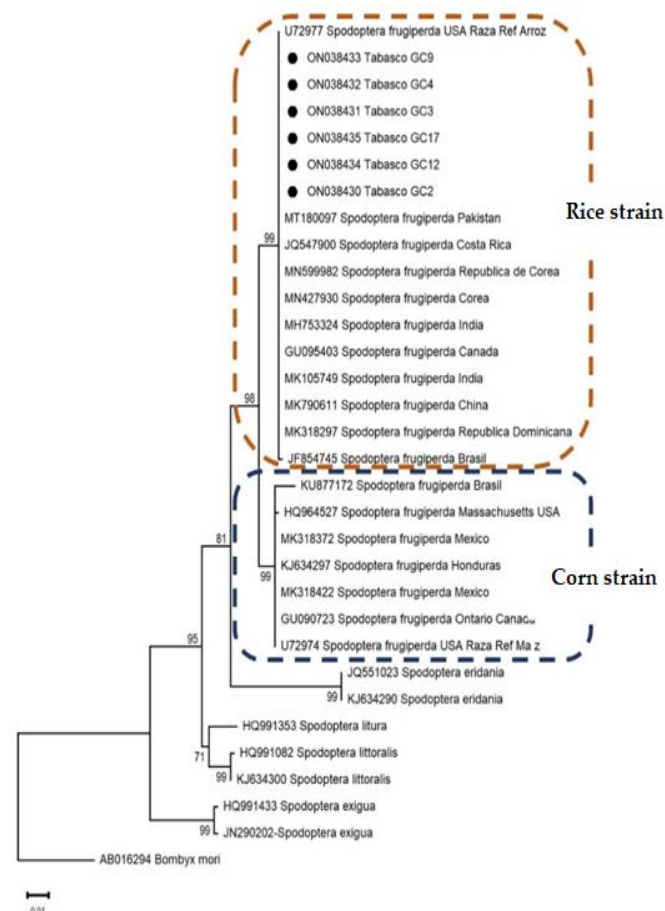


Figure 1. Phylogenetic tree of *Spodoptera frugiperda* in Tabasco, Mexico. Cytochrome c oxidase subunit 1 (COI) sequences from five species of Lepidoptera: Noctuidae were used to develop the tree in Figure 1, while *Bombyx mori* was used as an outgroup. The maximum likelihood method (ML) and Kimura's two-parameter model (K-2) (Kimura, 1980) was used to infer the evolutionary history. The tree with the highest log probability (-1,832.16) is shown here. The percentage of trees with clustered associated taxa can be found next to the branches. The first trees were developed in order to conduct the heuristic search, applying the Neighbor-Joining method to a pairwise distance matrix, calculated with the maximum composite likelihood (MCL) approach. The numbers in the nodes indicate bootstrap values (>50%, 1,000 replicates). GenBank accession numbers are shown before the name of each species. The bullet points indicate the samples collected in Tabasco for this study. The scale bar measures substitutions per site. The Clustal W software was used to align the nucleotide sequences and MEGA 11 was used to perform the evolutionary analyses (Tamura *et al.*, 2021).

strains from corn (n=7) and rice (n=11). *S. littoralis* (Boisduval, 1833) (n=2), *S. eridania* (Cramer, 1782) (n=2), *S. exigua* (Hübner, 1808) (n=2), *S. litura* (Fabricius, 1775) (n=1), and *Bombyx mori* (Linnaeus, 1758) (n=1) were selected as outgroup (Table 1).

The sequences of the barcoding COI region of *S. frugiperda* trapped at INIFAP-CEHUI clearly group with the reference sequences of the rice strain (U72977) (Figure 1).

The maximum likelihood analysis supported the results of Hebert *et al.* (2010), who considered that COI gene barcoding was an effective identification tool. *Spodoptera frugiperda*

Table 1. Specimen data and GenBank accession numbers used in this study.

Species	Reference	GenBank	Gene	Country
<i>Bombyx mori</i>	Kim <i>et al.</i> , (2000)	AB016294	COI	
<i>Spodoptera exigua</i>	Zahiri <i>et al.</i> , (2017)	JN290202	COI	U.S.
<i>Spodoptera exigua</i>	Zahiri <i>et al.</i> , (2017)	HQ991433	COI	Pakistan
<i>Spodoptera frugiperda</i>	This study	ON038430	COI	Mexico
<i>Spodoptera frugiperda</i>	This study	ON038431	COI	Mexico
<i>Spodoptera frugiperda</i>	This study	ON038432	COI	Mexico
<i>Spodoptera frugiperda</i>	This study	ON038433	COI	Mexico
<i>Spodoptera frugiperda</i>	This study	ON038434	COI	Mexico
<i>Spodoptera frugiperda</i>	This study	ON038435	COI	Mexico
<i>Spodoptera littoralis</i>	Ashfaq <i>et al.</i> , (2017)	HQ991082	COI	Pakistan
<i>Spodoptera littoralis</i>	van de Vossenber & van der Straten, (2014)	KJ634300	COI	Zimbabwe
<i>Spodoptera litura</i>	Ashfaq <i>et al.</i> , (2017)	HQ991353	COI	Pakistan
<i>Spodoptera eridania</i>	Zahiri <i>et al.</i> , (2017)	JQ551023	COI	Costa Rica
<i>Spodoptera eridania</i>	van de Vossenber & van der Straten, (2014)	KJ634290	COI	Surinam
<i>Spodoptera frugiperda</i>	Maas & Sanjur, (1996)	U72974	COI	U.S.
<i>Spodoptera frugiperda</i>	van de Vossenber & van der Straten, (2014)	KJ634297	COI	Honduras
<i>Spodoptera frugiperda</i>	Gilligan <i>et al.</i> , (2019)	MK318422	COI	Mexico
<i>Spodoptera frugiperda</i>	Ratnasingham & Hebert, (2013)	GU090723	COI	Canada
<i>Spodoptera frugiperda</i>	Zahiri <i>et al.</i> , (2017)	HQ964527	COI	U.S.
<i>Spodoptera frugiperda</i>	Gilligan <i>et al.</i> , (2019)	MK318372	COI	Mexico
<i>Spodoptera frugiperda</i>	-	KU877172	Mitogenome	Brazil
<i>Spodoptera frugiperda</i>	-	JF854745	COI	Brazil
<i>Spodoptera frugiperda</i>	Gilligan <i>et al.</i> , (2019)	MK318297	COI	Dominican Republic
<i>Spodoptera frugiperda</i>	-	MK105749	COI	India
<i>Spodoptera frugiperda</i>	Hebert <i>et al.</i> , (2010)	GU095403	COI	Canada
<i>Spodoptera frugiperda</i>		MH753324	COI	India
<i>Spodoptera frugiperda</i>	Jing <i>et al.</i> , (2020)	MK790611	COI	China
<i>Spodoptera frugiperda</i>	-	MN427930	Mitogenome	Korea
<i>Spodoptera frugiperda</i>	-	MT180097	COI	Pakistan
<i>Spodoptera frugiperda</i>	-	MN599982	Mitogenome	South Korea
<i>Spodoptera frugiperda</i>	-	JQ547900	COI	Costa Rica
<i>Spodoptera frugiperda</i>	Maas & Sanjur, (1996)	U72977		U.S.

—which encompasses the “rice” and “corn” strains, based on host plant preferences— does not show any morphological differences (Nagoshi *et al.*, 2015; Pashley, 1986).

Therefore, in this study molecular identification methods were more accurate than morphological identification (Jing *et al.*, 2020). Although the strains are morphologically indistinguishable, they have different genetic markers, with polymorphisms in the mitochondrial COI gene among the best characterized. Nagoshi *et al.* (2015) mention a deficiency in the identification of the distribution pattern of the FAW CS in Central America and Mexico —which is far less significant regarding the RS.

Through the phylogenetic comparison of the COI region, the six sequences used in this study are grouped into the *S. frugiperda* RS clade (Figure 1); these results differ from those previously reported in Mexico. An earlier study used two segments of the mitochondrial COI gene to confirm that populations from four FAW colonies in Mexico (Durango, Sinaloa, Tamaulipas, and Chiapas) belong to the *S. frugiperda* CS clade (Nagoshi *et al.*, 2015).

The barcodes obtained from our samples were compared with public barcodes in GenBank, revealing that the isolates from Chontalpa, Tabasco, genetically align with FAW strains from other latitudes and with the reference RS GenBank No. U72977. The alignment of the sequences used in this study shows a 100% match with those reported for the RS in India (Swamy *et al.*, 2018); the CS in China (Jing *et al.*, 2020), Korea (Kim *et al.*, 2021), and Pakistan (Lalramnghaki *et al.*, 2021); sorghum in India (GenBank No. MH753324); sugarcane in Malawi (Kasambala Donga and Meadow, 2018); and *chili* spp. in the Dominican Republic (Gilligan *et al.*, 2019), Costa Rica (GenBank No. JQ547900), and Canada (GenBank No. GU095403).

This is the first genetic study characterizing the FAW in Tabasco by using a region of the COI gene. Strain identification of *S. frugiperda* is necessary for management purposes, as each strain may require a different control method. Moreover, some *S. frugiperda* populations show extraordinarily high resistance to insecticides such as pyrethroids, organophosphates, and diamides (Kim *et al.*, 2021).

Having established the presence of the *S. frugiperda* RS in the Chontalpa area of Tabasco, further studies are required to identify the *S. frugiperda* CS, in order to achieve an effective and efficient control. The latter must consider these species' resistance to insecticides and the distribution of both strains. The two strains breed continuously from the southern US to northern Argentina, and both are found further north and further south during summer and fall, since they are seasonally breeding migrant populations, but cannot tolerate <0 temperatures (Cock *et al.*, 2017).

The Food and Agriculture Organization (FAO) of the United Nations recommends using pheromone traps to determine the incidence and severity of *S. frugiperda* (Prasanna *et al.*, 2018). Accurate identification of pest species is crucial to the effectiveness of pheromone traps as a monitoring tool. However, Unbehend *et al.* (2014) report that males of the corn and rice strains had diverse responses to different combinations of synthetic pheromones in various geographic regions.

The RS seems to colonize corn, sweet corn, and sorghum (Swamy *et al.*, 2018). However, although the strains are named after their preferred host plants, there is no certainty as

to the host specificity of each one. Both the RS and the CS feed on corn during the same cultivation period (Nagoshi *et al.*, 2007), a phenomenon which was already reported by Pashley (1986), who differentiated both strains by food preference, although not the specificity of the host plant.

Therefore, DNA barcoding will facilitate the identification of pests in the country and support taxonomic work where necessary —*i.e.*, for species requiring specialized studies to enable identification and lacking a reference barcode, as well as for species that require differentiation as a consequence of their morphologically similar taxa.

CONCLUSIONS

The DNA barcode approach (barcoding) facilitated the identification of the *S. frugiperda* RS in southern Mexico. Confirmation of the presence of RS in the study area is of the utmost importance, since this strain of *S. frugiperda* can infest sugarcane, rice, and corn crops. Considering the year-round availability of host plants and the voracious nature of *S. frugiperda*, this species can potentially become a dangerous pest in sugarcane- and rice-growing areas of Tabasco, if no effective measures are taken to control its spread.

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Use of equine chorionic gonadotropin (eCG) in Mashona heifers, under a J-Synch synchronization protocol, and its effect in pregnancy rate

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ABSTRACT

Objective: To evaluate the J-Synch protocol with two eCG doses in beef heifers during the summer in northeastern Mexico.

Design/Methodology/Approach: 218 heifers (109/treatment) with a body weight of 350 ± 12.1 kg were used. A J-Synch protocol was applied in order to evaluate two eCG doses: T1 (250 IU) and T2 (300 IU). Subsequently, the total number of heifers from both treatments were inseminated at a fixed time (FTAI): 72 h after removing the device.

Results: No significant differences ($p > 0.05$) were recorded in the estrous percentage at first service (T1=91%; T2=96%) and in the repeating heifers (T1=25.2%; T2=19.2%). The insemination technician and coat color variables impacted the estrous percentage in repeating heifers. The pregnancy percentage at first service was 64.8% and 70.1% for T1 and T2 ($p > 0.05$), respectively. The insemination technician had a variable effect on the pregnancy percentage, from 63.4% (the best-qualified technician) to 48.6% (the technician who obtained the lowest percentage).

Study Limitations/Implications: The pregnancy rate in beef heifers will depend mainly on the experience and skill of the insemination technician.

Findings/Conclusions: The same results were obtained regarding the presence of estrous and pregnancy in beef heifers during the summer season, either with 250 or 300 IU of eCG.

Keywords: Mashona heifers, estrous, artificial insemination, breeding season, GnRH.

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INTRODUCTION

Worldwide cattle raising faces a complex outlook due to the climate change that mainly affects the semi desertic regions, as a result of the lack of rainfall. This phenomenon severely limits the productivity of cow-calf production systems. Consequently, breeds with medium to low body weight, with low management requirements, great adaptation to extreme weather, and which tolerate heat stress during the summertime have been introduced (Ferreira *et al.*, 2011; Fernandez-Novo *et al.*, 2020). One such breed is Mashona (*Bos taurus*), an African breed with black, brown, and reddish coat. In semi desertic regions

with a high level of solar radiation, this breed could face reproductive issues, mainly related to pregnancy rates.

Anzures-Olvera *et al.* (2019) reported that dark coat colors absorb more solar radiation, while Bertipaglia *et al.* (2018) recorded an outward transfer of heat energy from the coat, impacting body temperature. Consequently, pregnancy rates diminish when heifers suffer heat stress 42 days before and 40 days after being artificially inseminated (Jordan, 2003).

In cow-calf production systems, genetic improvement through artificial insemination and estrous and ovulation synchronization programs have become widespread throughout the years (Bó and Baruselli, 2014; Yáñez-Avalos *et al.*, 2021). Modifications to the estrous and ovulation synchronization protocols have helped to correct the absence of estrous and ovulation and have increased pregnancy and calving rates in beef cattle (Dias *et al.*, 2009). One of the most outstanding improvements is fixed-time artificial insemination (FTAI), which includes a progesterone-based hormonal treatment and the administration of estradiol benzoate (EB) at the start of the protocol. The intravaginal device is removed on the eighth day and a synthetic prostaglandin is applied, along with estradiol cypionate. Alternatively, EB is administered 24 h after the FTAI —on average, 56 h after the device is removed (Bó and Cedeño, 2018; Baruselli *et al.*, 2018). However, during the last decade, estrous synchronization protocols have been developed, focusing on beef heifers (Colazo *et al.*, 2017). These protocols face the following limitations: an extended proestrus stage; an inadequate development of the follicle diameter or the age of the ovarian follicle (Menchaca *et al.*, 2015); and a corpus luteum that can produce enough progesterone which can support pregnancy (Aréchiga-Flores *et al.*, 2019; Bó *et al.*, 2019). These principles were the basis for the development of the J-Synch protocol, a short 5-6 d methodology (Motta *et al.*, 2016; Macmillan *et al.*, 2020), during which equine chorionic gonadotropin (eCG) is administered at the time of the removal of the intravaginal device (Motavalli *et al.*, 2017), replacing the estradiol cypionate and the administration of GnRH, 72 h after the vaginal device is removed (Menchaca *et al.*, 2015; Reineri *et al.*, 2020; Zwiefelhofer *et al.*, 2021; Núñez-Olivera *et al.*, 2022).

Under the J-Synch protocol, the eCG will boost the increase of the circulating estradiol concentrations (De la Mata *et al.*, 2015; De la Mata *et al.*, 2018), the diameter of the ovarian follicle (Souza *et al.*, 2009; Pessoa *et al.*, 2016), and the progesterone concentration after the ovulation in beef heifers (Sales *et al.*, 2016; Ferraz *et al.*, 2019; Núñez-Olvera *et al.*, 2020). Based on this scheme, 300 IU (Núñez-Olvera *et al.*, 2020; Yanet-Avalos *et al.*, 2021) or 500 IU (Mahdavi-Roshan *et al.*, 2020) doses have been used to induce the ovulation of the codominant follicle. These eCG doses have been used in medium and large size cows; however, their effect in medium to small size heifers (such as Mashona cows) has not been evaluated yet. Therefore, the objective of this study was to determine the effect of two eCG doses on the estrous and the pregnancy percentage of 13-month-old heifers, during the summer in northeastern Mexico. Recent studies carried out with dairy cattle established that the use of two eCG doses (0 and 300 IU) does not affect the follicle dynamic or the pregnancy rate (Sanz *et al.*, 2022); consequently, this study proposes the hypothesis that using two doses of eCG (250 and 300 IU) will have a similar effect on the estrous and the pregnancy rate of beef heifers.

MATERIALS AND METHODS

The experimental work was carried out in the Rancho Río Salado, located in the municipality of Vallecillo, Nuevo León, México (26° 53' 25.1" N and 99° 52' 15.6" W), at 274 m.a.s.l., with a 580-mm mean annual precipitation. The experiment was developed from June to August 2021, during the summer in northeastern Mexico, when the average temperature was 35.3 ± 2.1 °C.

Animal handling

The study was carried out according to the NOM-062-ZOO-1999 official standard, complying with the care and welfare of the animals during all the stages of the 12-week research. Eighteen-month-old heifers (n=218) from the Mashona (*Bos taurus*) breed were used for the experiment; on a scale from 1 to 9, they had a body condition (BC) of sex and body weight (BW) of 350 ± 22.5 kg. All the heifers grazed in a marvel grass (*Dichanthium annulatum*) prairie, and they also had free access to mineral salt (Fosminsal 9%, Agronutrientes del Norte, General Escobedo, N.L., Mexico).

Treatments

Before the establishment of the experimental work, a reproductive evaluation was carried out using an Eco2 real time ultrasound with a 7.5 MHz transrectal probe (Sonoscape, USA). The aim of this evaluation was to exclude pregnant animals or animals with poor development of their reproductive system. Consequently, out of 232 animals, n=218 were available for the study. In order to assign the treatments and to improve the artificial insemination (AI) process, the heifers were randomly divided into two groups: Group I=114 heifers and Group II=104 heifers.

Estrous synchronization and ovulation

The J-Synch protocol was applied to a total of 218 heifers, which were inseminated a week apart, according to the group to which they had been assigned. The protocol consisted of the application of 2 mg of EB (Internacional Prode, Jalisco, Mexico) and a Dispolcel Max intravaginal device (ID) (Internacional Prode, Jalisco, Mexico), with 1.2 g of synthetic progesterone on day 0. The device was removed on day 5 and 0.15 mg of cloprostenol (DC) (Internacional Prode, Jalisco, Mexico) was applied. The heifers of each group received one of the two eCG treatments: T1=250 IU and T2=300 IU. The hormone was applied at the moment of the removal of the intravaginal device (Figure 1). Regardless of the eCG treatment, all the heifers were subjected to fixed-time artificial insemination (FTAI), 72 h after the removal of the device; 0.5-mL straws of frozen semen from a bull of the same breed and of proven fertility were used for the FTAI. The heifers were inseminated by four experienced technicians (two technicians per group).

Each heifer received 10.5 µg of GnRH (Buserelin-acetate, Biogenesis Bago, Mexico) at the moment of the AI. In order to guarantee a 72-h AI within each group (consisting in an average of 25-50 heifers), the device was inserted and removed at 2 h intervals. The same intravaginal devices of the first service were used for the resynchronization of the estrous. The devices were washed, disinfected (with Antibenzil[®], Altamirano,

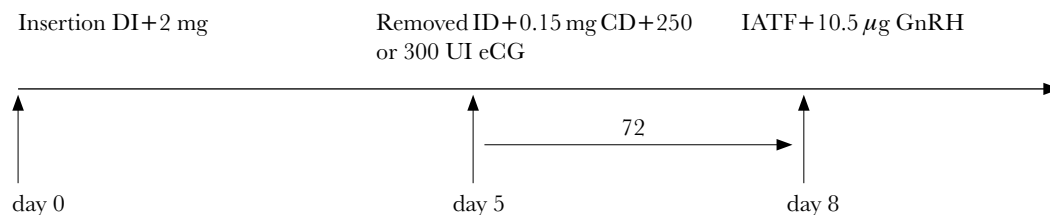


Figure 1. Arrangement of the J-Synch protocol used for the estrous synchronization and the ovulation, applied to Mashona heifers during the summer in northeastern Mexico. ID: intravaginal device; DC (CD): cloprostenol; FTAI (IATF): fixed-time artificial insemination; eCG: equine chorionic gonadotropin; GnRH: gonadotropin-releasing hormone.

Mexico), and dried at room temperature. The devices were reinserted 13 days after the first service and were removed 7 days later. In order to detect the estrous, the base of the tail of the heifers was painted; afterward, the heifers were divided into groups depending on the time that the estrous was detected. Heifers were inseminated 12 h later. The intravaginal devices that fell at the removal, during the insertion and the reinsertion periods, were counted. The devices that were not found inside the vagina at the moment of the removal were considered lost.

A Chi-square (χ^2) test was used to determine the discrete variables—such as percentage (%) of estrous, repeating heifers, pregnancy at the first and second service, and devices lost during the first and second service. A lineal model was used to determine the removal-artificial insemination interval (h) and the time used to inseminate the heifer (s). The factors evaluated by this model were the eCG=250 and 300 IU doses, the group number (I and II), the insemination technician, and the color of the coat of the heifer (black, brown, and reddish). The SPSS software, version 22, was used.

RESULTS AND DISCUSSION

Table 1 shows that there were not differences regarding the percentage of estrous and the eCG doses applied, and the time after the removal of the devices, as well as the time required to carry out the AI. Regarding the evaluated factors, only the inseminator technician and the color of the coat impacted the percentage of the repeating heifers in the second service. The A and F technicians obtained a >30% estrus. These technicians were in charge of inseminating the first group of heifers. Consequently, Table 1 shows that Group I had a higher percentage of estrus than Group II ($P<0.001$). Additionally, regarding the coat of the heifers, brown coat heifers recorded the highest estrus percentage, doubling the percentage (36.9%) obtained by the reddish and black heifers. Regarding the removal-AI period, the parameter of the J-Synch protocol was fulfilled: in average the insemination was carried out 72 h after the removal of the intravaginal device. Table 1 also shows the differences in the pregnancy % of the first and second services related to the insemination technician. The best insemination technician recorded a 14.8% difference in the pregnancy %, compared with the technician that recorded the lowest pregnancy % ($P<0.05$). The other factors did not record statistically significant differences ($P>0.05$).

Table 1. Percentage of estrus during the second service, average time of insemination, time required to inseminate each heifer, and total pregnancy % of the first and second services (mean \pm SE).

Effect	Repeater heifers (% / estrous)	Interval removal-AI (h)	Time AI (s)	Pregnancy (%)
Inseminator technician				
A	13/35 (37.1) ^a	73.5 \pm 0.2	80.8 \pm 15.5	48.6 (17/35) ^b
B	4/58 (6.8) ^b	72.3 \pm 0.1	90.4 \pm 14.3	59.5 (34/58) ^{ab}
C	32/79 (40.5) ^a	73.3 \pm 0.1	88.1 \pm 10.5	51.9 (41/79) ^b
D	2/47 (4.2) ^b	72.4 \pm 0.2	77.9 \pm 17.8	63.4 (30/47) ^a
Treatment: eCG				
250 IU	28/111 (25.2)	72.9 \pm 0.1	81.5 \pm 10.2	64.8 (72/111)
300 IU	21/107 (19.2)	72.8 \pm 0.1	87.0 \pm 10.6	70.1 (75/107)
Color coat:				
Reddish	3/19 (15.7) ^b	72.7 \pm 0.1	74.3 \pm 17.0	63.1(12/19)
Brown	17/46 (36.9) ^a	72.8 \pm 0.1	88.2 \pm 13.0	63.0 (29/46)
Black	29/153 (18.9) ^b	73.0 \pm 0.0	90.2 \pm 5.3	59.4 (89/153)
Lot:				
I	45/114 (39.4) ^a	73.4 \pm 0.0	84.6 \pm 9.3	60.5 (69/114)
II	4/104 (3.8) ^b	72.3 \pm 0.1	84.1 \pm 11.4	67.3 (70/114)

^{a,b} The subscript of letters inside the same column are statistically different ($P < 0.05$).

Table 2 indicates the values of the lost device percentage, both for the first insertion and the reinsertion of the devices. No significant differences were recorded ($P > 0.05$). A high number of intravaginal devices were lost during the reinsertion of the devices, regardless of the groups or the color of the coat.

In this study, the hypothesis was that 250 or 300 IU of eCG will not result in differences in the estrus and pregnancy % of heifers, applying a J-Synch protocol. Given the lack of differences between both doses, the Mashona breed would respond to a lower dose. Remarkably, the pregnancy percentage was higher than the results reported by Macmillan *et al.* (2020), who used a J-Synch protocol with 300 IU of eCG and obtained a 48.7% pregnancy. However, this percentage can be the result of the presence of the corpus luteum at the moment when the eCG was applied.

Table 2. Loss of the intravaginal devices inserted in Mashona heifers, during the first and second services, using FTAI with a J-Synch protocol ($P > 0.05$).

	Losses device first insertion (%)	Losses device reinsertion (%)
Lot		
I	9/114 (7.8)	11/114 (9.6)
II	12/104 (11.5)	18/104 (17.3)
Color coat		
Reddish	1/19 (5.2)	3/19 (15.7)
Brown	3/46 (6.5)	2/46 (4.3)
Black	17/153 (11.1)	24/153 (15.6)

The pregnancy rate was 20% lower than the results for Angus heifers recorded by De la Mata *et al.* (2015). In our study, reducing the time of insertion of the progesterone device extended the proestrus period, which could have improved the pregnancy %. An additional factor of improvement is to carry out the insemination 12 h after the estrus is detected. This study provides another alternative: a fixed time insemination, carried out 72 h after the removal of the device (Bó *et al.*, 2016).

In this regard, the impact of eCG on the pregnancy rate seems to extend the duration of the proestrus, improving the ovarian follicle diameter and the estradiol content, as well as the luteum function. Consequently, insemination 72 h after the removal of the device increased the pregnancy %, which matched the results of Nuñez-Olvera *et al.* (2020) and Sanz *et al.* (2022). In contrast to heifers, the use of eCG with or without the estrus did not change the pregnancy rate of adult cows (Mion *et al.*, 2019). The detection of the estrus during the first service was not a limitation in this study; however, the second service recorded low estrus percentages. This phenomenon could be the result of the adaptation of this breed to the negative effects of heat stress. Most of the studies about heifers have been carried out in favorable environments. However, this study was carried out during summer, with a range of temperatures from 30 °C to 35 °C. The above-mentioned conditions match the findings of Anzures-Olvera *et al.* (2019), who carried out their study in similar areas and found that the dark color of the Holstein heifers did not impact their reproductive yield and, instead, the temperature conditions affected milk production. The heat stress that prevailed during summer could have jeopardized the potential quality of the oocytes. Nevertheless, the breathing rate and the rectal temperature of the dark color coat heifers tend to raise, increasing the skin temperature and reducing the quality of the oocytes (Ferreira *et al.*, 2011). However, these results are different from those reported by Bertipaglia *et al.* (2018), who found that cows with <2 mm dark pigmentation recorded 0.3 conception services less than cows with >3 mm dark pigmentation areas. Although these studies are the first to be conducted using heifers under a J-Synch protocol, the pregnancy results of this study are acceptable, considering that they were carried out during a summer of extreme temperatures in northeastern Mexico. Nevertheless, further studies are required to determine if the J-Synch protocol has a growing rate that matches the dominant follicle after ovulation. That rate could result in a higher progesterone concentration and, therefore, a higher pregnancy percentage, particularly in heifers exploited under extreme summer conditions (Bó *et al.*, 2018).

This study determined that the insemination technician plays an important role in the process. Four expert technicians carried out the AI on the two groups, with 14 days of difference between procedures. The pregnancy rate difference between the technician who recorded the highest pregnancy % and the technician who recorded the lowest percentage was 16%. These differences could be the result of the amount of knowledge and the animal handling experience and, above all, the years of experience that the best technician has, given that the technician constantly puts the procedure in practice, manipulating the reproductive system of the animals. In the particular case of the Mashona heifers, we found out that the structure of the cervical canal obstructs the introduction of the insemination gun (Anzar *et al.*, 2003). García-Ispuerto *et al.* (2007) reported that the inseminator technician

contributed to the fertility improvement of dairy cows, because 23% of the technicians were experts and professionals. In our study, 25% of the inseminator technicians achieved a pregnancy improvement of at least 15%. Additionally, the percentage of intravaginal devices lost during the insertion and the reinsertion was higher than the figure reported by Hernández *et al.* (2008) in their study with adult Brangus (2.3%). The number of devices lost in our study was higher than the number recommended by Islam (2011) for cattle (<5%). This high percentage of lost intravaginal devices can mainly result from the narrow space in which the device can be inserted into the heifers: a third part of the device remained outside the vagina.

CONCLUSIONS

The J-Synch protocol is a reproduction management alternative to synchronize the estrus and the ovulation of heifers under the summer conditions of northeastern Mexico. Two-hundred fifty IU of eCG can be used without compromising the pregnancy rates of heifers, considering that the skill of the insemination technician will play an important role in the pregnancy in beef cattle. In this study, the Mashona breed obtained appropriate pregnancy rates regardless of the color of the coat of the heifers.

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Corn price volatility and producer income protection

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ABSTRACT

Objective: To estimate the risk indicator of the future price of yellow corn #2 at the Chicago Futures Exchange (USA) regarding the spot price of white corn in the main producing regions in Mexico through the financial volatility indicator.

Methodology: The research used the returns of the monthly time series corresponding to the spot price of white corn from January 1998 to December 2020, considering five producer-consumer regions of Mexico and the future price of yellow corn #2 as listed on the Chicago Stock Exchange. To quantify volatility, the generalized autoregressive conditional heteroskedasticity model of order (1,1) was estimated.

Results: The yellow corn #2 volatility indicator was 0.9870 (future price). In the case of the spot price of white corn in Mexico, the volatility was 0.7977 for the national price, 0.3385 for the central region, 0.3206 for the western region, and 0.0078 for the southeast region.

Implications: The high volatility of yellow corn #2 (close to unity) shows that the international market for this commodity is riskier than the national market or regional markets in Mexico.

Conclusions: The national white corn market proved to be riskier than the west, center, and southeast regional markets, which have a higher volatility indicator.

Keywords: futures market, autoregressive conditional heteroskedasticity, risk coverage, supply, agricultural policy.

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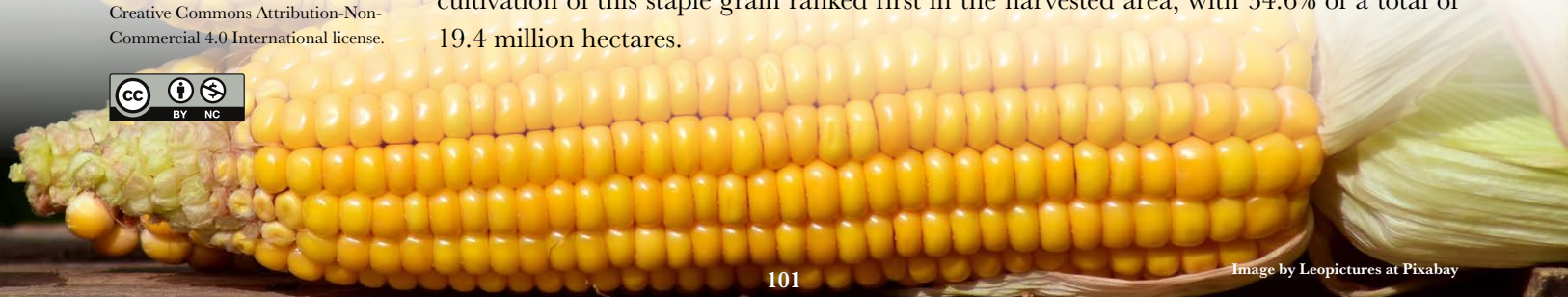
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INTRODUCTION

In Mexico, the promotion of corn production has been a priority sectoral policy, because it is the fundamental grain in the population diet. The guaranteed price of this crop was maintained until 1999, the year in which the Compañía Nacional de Subsistencias Populares (CONASUPO) was liquidated. This policy was in force for 15 years (1993-2007), to face the total opening to free trade and international competition of this grain with the country's trading partners (Ortiz and Montiel, 2017; Valdes, 2018).

During the 2000-2019 period, corn imports registered a 5.5% average annual growth rate. According to the Agencia de Servicios a la Comercialización y Desarrollo de Mercados Agropecuarios (ASERCA, 2020), between 2011 and 2019, yellow corn and white corn accounted for an average of 91.2% and 8.8% of total imports, respectively. In 2019, the cultivation of this staple grain ranked first in the harvested area, with 34.6% of a total of 19.4 million hectares.



Given the commercial opening of applied agriculture in the 1983-2018 period, Zahniser *et al.* (2019) and Motamed *et al.* (2008) point out that the different regions of Mexico are not equally integrated with the United States market. Therefore, white corn prices throughout Mexico can change along, but in different degrees, with the price of yellow corn #2 in the United States. The Apoyos y Servicios a la Comercialización Agropecuaria (ASERCA) decentralized public organization was created in 1991. ASERCA implemented and monitored part of the public policies that benefited producers, strengthening the development of the sector, agricultural profitability, and producers' income. In addition, it solved problems that arose when producers stopped receiving support from CONASUPO (Godínez, 2006; Varangis, Larson, and Anderson, 2002).

In the federal administration of the 2000-2006 period, ASERCA's coverage was extended to support five crops (cotton, beans, wheat, sorghum, and soybeans) in face of the commercial opening. It also helped corn growers to purchase futures contracts for yellow corn #2, which are quoted on the Chicago Futures Exchange, under the argument that white and yellow corn are interchangeable with each other in the offer. Support through futures contracts ended in 2018, because in 2019 ASERCA's budget was reduced by 88.2 %.

The acquisition of coverage contracts in the futures market of the Chicago Stock Exchange is subject to high speculation; therefore, its prices register high volatility, as can be deduced from the works of Engle (1982) and Bollerslev (1986). In addition, the magnitude of the volatility of the yellow corn #2 future price (transmitted to the spot price of white corn in Mexico) is not well known. Therefore, if Mexico is the main importer of yellow corn from the United States, it is important to provide elements for decision-making in the design of white corn agri-food policies in Mexico. The objective of this research was to estimate the magnitude at which the risk of the yellow corn #2 futures price in the Chicago Futures Exchange is passed on to the spot price of white corn in the main producer-consumer regions of Mexico. This magnitude was determined through the financial volatility indicator for the 1998-2020 period, to analyze the effect of coverage on the income of producers as a tool to protect the income of surplus white corn producers.

MATERIALS AND METHODS

In the study, four white corn producing regions in the country were considered: 1) the western region that includes Sinaloa and Jalisco; 2) the central region that groups Mexico City and the State of Mexico; 3) the southeast region made up of Chiapas and Yucatán; and 4) a "national" region that includes Chiapas, Chihuahua, Mexico City, Durango, Jalisco, State of Mexico, Nuevo León, Sinaloa, Tamaulipas, Yucatán, and Zacatecas.

Variables definition

Table 1 shows the definition of the main variables used in the study.

The sampling period of the study covered 22 years, from January 1998 to December 2020, obtaining a sample of 264 observations. The monthly series of price data for yellow corn #2 listed on the Chicago futures market was obtained from the Economic Research Service (ERS-USDA, 2021), while the price of white corn was obtained from the Servicio

Table 1. Definition of study variables.

Variable	Description	Units
PBOCC	Price of white corn Western Region	USD/ton
PBCEN	Price of white corn Central Region	USD//ton
PBSTE	Price of white corn Southeast Region	USD//ton
PBNAL	National white corn price	USD//ton
PAMBC	Price of yellow corn #2 listed on the futures market of the Chicago Stock Exchange	USD//ton

Note: USD=United States dollar; t=metric ton. Source: table developed by the authors.

Nacional de Información e Integración de Mercados of the Secretaría de Economía (SNIIM, 2021).

Non-stationarity contrast tests

The methodology used to determine if the time series are stationary was the Dickey-Fuller unit root (DF) contrast test (Brooks, 2019). In the case of the financial concept of volatility analysis, the so-called performance or profitability was used. Brooks (2019) indicates that, in financial analysis, it is better to express asset prices as simple price performance or profitability. Given that the prices of certain commodities (*e.g.*, coffee and corn) behave like the prices of financial or similar assets, their residuals also have high variability and heteroskedasticity.

RESULTS AND DISCUSSION

Descriptive statistics

To quantify the volatility of the white and yellow corn prices, these were transformed into yields or simple profit, based on the proposals of Brook (2019) and Pérez (2006). Table 2 shows the descriptive statistics of the four series of the white corn profitability and the series of the yellow corn #2 profitability.

Unit root test

The values of (τ) were established using the augmented Dickey Fuller test (ADF) for unit root, including only the intercept in the regression equation. The values of τ for the profitability of the five-time series were higher than the critical values for the 1, 5, and 10%

Table 2. Descriptive statistics of the profitability of white and yellow corn.

Statistics	PMBOCC	PMBCEEN	PMBSTE	PMBNAL	PAMBC
Average	0.3034	0.3046	0.3126	0.3052	0.8032
Maximum	45.0861	22.2119	23.3661	22.6043	89.9500
Minimum	-31.6218	-18.1686	-16.5859	-17.2037	-41.1700
Standard deviation	6.4303	4.3940	5.5306	4.3429	12.1155
Symmetry coefficient	1.1004	0.4160	0.6037	0.5113	2.0284
Kurtosis	13.3615	6.6185	5.7773	7.7817	17.5227

Source: table developed by the authors.

confidence levels; consequently, all the time series were stationary. The abovementioned GARCH (1,1) model was used in the research. For the analysis of the volatility of the series of interest, the following two equations were estimated: the mean regression equation and the variance equation that corresponds to GARCH itself (1,1).

Table 3 shows the estimated results of both equations for each of the white and yellow corn series. This table also includes the price volatility of these corns, which is considered a measure of the market risk that their buyers and sellers must face. They must also try to minimize or transfer the said risk to other market agents, through financial instruments (*e.g.*, coverage or insurance premiums).

Table 3. Estimation of the volatility of corn with the GARCH model (1,1).

Variable	Equation / Parameter	Coefficient	Standard error	Z-value
RPMAMRD	Equation of the mean:			
	α	-0.04335	0.23595	-0.18371
	Variance equation:			
	α_0	0.41254	0.11351	3.63451
	α_1	0.04216	0.01049	4.02090
RPMBRDNAL	Ecuación de la media:			
	α	-0.31168	0.29382	-1.06077
	Variance equation:			
	α_0	5.95383	1.25095	4.75929
	α_1	0.26910	0.05640	4.77171
RPMBRDOCI	Equation of the mean:			
	α	-0.10931	0.41122	-0.26582
	Variance equation:			
	α_0	29.56558	9.45214	3.12793
	α_1	0.23223	0.07434	3.12376
RPMBRDCEN	Equation of the mean:			
	α	-0.08040	0.25434	-0.31609
	Variance equation:			
	α_0	16.00216	2.42771	6.59146
	α_1	0.41661	0.09442	4.41254
RPMBRDSTE	Equation of the mean:			
	α	-0.24175	0.42394	-0.57023
	Variance equation:			
	α_0	45.37773	116.81590	0.38846
	α_1	0.01607	0.03560	0.45159
	β	-0.00825	2.57997	-0.00320

Source: table developed by the authors.

The coefficient column shows the estimated values for the parameters of the equation of the mean, which are assumed to be shaped like a random walk with drift. In the case of the estimated parameters for the GARCH (1,1) model, the restrictions ($\alpha_1 > 0$ and $\beta > 0$) are fulfilled. The interpretation corresponding to GARCH (1,1) is $\alpha_1 + \beta = 0.2691 + 0.528551 = 0.798$ —that is, the sum of the coefficient of the ARCH effect and the parameter of the conditional variance with a one-period lag. This implies that the price of white corn for the following month is highly dependent on the price of the previous period, showing a high persistence of volatility (particularly, of the national corn price) and therefore a greater risk.

In the southeast region (RPMBSTE), both the parameter of the ARCH effect (α_1) and the conditional variance (β) were not statistically significant. Likewise, the magnitude of volatility ($\alpha_1 + \beta = 0.0078$) does not affect white corn.

For their part, Ortiz and Montiel (2017) used the multivariate stochastic volatility analysis to show that the corn price in the futures market during the 2007-2012 period was not strongly related to the prices recorded in some states of the country. Therefore, they conclude that, despite the increased use of white corn, the coverage provided by the ASERCA program has failed to properly fulfill its purpose: protecting the income of Mexican farmers who plant this grain.

Similarly, the present study found out that the average price of white corn in Mexico has a high volatility that, given its magnitude (0.798), tends to persist over time. The southeast region (Chiapas and Yucatán) is not affected by the said volatility. Meanwhile, Echánove (2011) highlights that the government implemented support programs since the 1990s, including contract farming, whose purpose was to provide security, both to producers and buyers, in the grain commercialization sector. In 1996 producers (mostly of white corn) began to use the program to acquire stock instruments on the Chicago Stock Exchange, with yellow corn #2 as their underlying. The coverages operated by ASERCA involve a position in the futures market opposed to the position of the participant in the domestic spot market. Consequently, any loss in the spot market is compensated with the coverage in the futures market (Ortiz and Montiel, 2017) and, at no point in time, do the producers—who are protected with the coverage signed with ASERCA—lose their monetary income as a result of the fall in the price implied in the high volatility. When the volatility implied a higher price than the one stipulated in the contract for the coverage acquired through ASERCA, the producers were not only protecting their income, but also obtained additional income from the difference in the prices of the contract and the yellow corn futures price. They only reimbursed ASERCA less than 50% of the amount which the said institution provided them to acquire the so-called base. In their analysis of the results of the volatility estimates, Ortiz and Montiel (2017) do not indicate how this type of risk would affect white corn producers in Mexico. At budget level, the official resources allocated to agriculture by contract are insufficient—as the budget allocated to the agricultural sector usually is (Echánove, 2011).

CONCLUSIONS

The profitability of the price of yellow corn #2—the underlying commodity of the coverage that ASERCA acquired to support white corn producers in the western region

(Sinaloa and Jalisco)— is highly volatile. The extreme closeness to the unity of this risk indicator implies a persistent volatility in the yellow corn #2 market and that the price of the next period depends almost entirely on the previous period. In the case of Mexico, the profitability of the white corn price in the national region has high volatility (0.798), which implies a high risk for white corn as the only national market. In conclusion, since the central and the western regions face a greater market risk and greater financial risk than the southeast region, their income could have greater variability and therefore greater risk. The need to design coverage mechanisms for corn producers will enable agricultural policy makers to develop programs and sectoral laws that encourage the national production and supply of this basic grain.

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Agronomic response of two experimental varieties of habanero chili in the application of band vermicomposting

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ABSTRACT

The habanero chili pepper has great productive potential in Mexico, with an annual growth of 12.5% in the planted area in the last five years.

Objective: was to evaluate the agronomic response of two experimental varieties of habanero chili called HNC-6 orange color and HCC-8 chocolate color, belonging to the Center for Training and Development in Seed Technology.

Methodology: the study was established under five doses of vermicompost, 0, 0.3, 0.6, 0.9 and 1.2 kg plant⁻¹ using a completely random design with a 2×5 factorial arrangement.

Results: the HNC-6 variety had a better agronomic response than HCC-8, its yield was 95% higher, the number of fruits per plant 84%, fruit length (FL) 25%, pericarp thickness 28%, fruit firmness 11%, and average fruit weight 4.7%. The HCC-8 variety exceeded the height of HNC-6 by 38% and the fruit diameter (FD) by 14%. The vermicompost doses exerted a similar effect on both varieties. In conclusion, the experimental variety HNC-6 showed a better agronomic response under the conditions tested. The vermicompost doses exerted a response effect and similar trend in the two experimental varieties of habanero pepper tested, the significant interactions found in FL, FD, and total soluble solids indicate that the application of vermicompost influenced the quality of the fruits in some aspects.

Conclusions: the main factors that determined the response of the crop are its genetic component in response to the environment and the degree of maturity of the vermicompost.

Keywords: *Capsicum chinense* Jacq. genotype, earthworm humus, quality, yield.

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INTRODUCTION

Habanero chili pepper (*Capsicum chinense* Jacq.) is a vegetable with high commercial potential in Mexico. It is considered as one of the spiciest chili peppers in the world since it presents the highest levels of burning aftertaste or pungency in Scoville units [1]. In the year 2021, Mexico reached a production value of \$441,205.73 million pesos, with

a surface of 1517 hectares planted, whose average yield was 17.99 t ha^{-1} , the main producing states are Sinaloa (305 hectares), Yucatán (264.72 hectares), Tabasco (274 hectares), Campeche (173 hectares), and Veracruz (116 hectares), where the main form of production is open-air and corresponds to 90.2% of the total production [2]. Producing habanero chili requires a high investment cost, although its value is generated due to its preference in domestic and exports markets; however, despite the profitability potential that it represents, there is a low technological level for its production process, which is reflected in the low harvest volumes [3].

On the other hand, the generation of new varieties will always be a priority objective for agriculture, and one of the tools to achieve it is genetic improvement, whose objectives is to select the best genotypes within the populations and to create new genotypes with previously defined characteristics. In Mexico, these activities are directed at increasing the productivity, the quality and the adaptability of cultivated species to current and future changes that are foreseen. In the case of habanero chili peppers, cultivars are sought with high levels of capsaicin and ascorbic acid, and it is considered that Yucatán is the center of genetic diversity of habanero chili pepper, so the potential for improvement is high [4].

Habanero chili pepper is one of the products that is demanded in large volumes globally, due to its high levels of capsaicin, which confer an exclusive added value to the fruits; therefore, producers aim at exports markets as their target business, although they are particularly strict with the management of inputs that are required in the production process [4]. Under this context, organic production is a development strategy that attempts to change the limitations in conventional production; it is founded not only on improving the soil and a promotion of the use of local inputs, but also in giving a higher added value to the final products, in addition to being perceived and promoted as healthy and safe for the environment [5]. It has been documented that organic fertilizers improve the soil quality and as consequence the environment, and consequently foster sustainable agriculture [6]. An example of this is vermicomposting, which is the process by which organic waste is decomposed through the synergic actions of earthworms and microbial communities [7], in addition to containing nutrients that are important in the growth and productivity of plants [8], and also substantially improve the chemical, biological and physical properties of the soil [9]. Therefore, the application of vermicompost can represent an alternative to help modify the management of agricultural inputs, promoting growth, productivity and profitability of the crops in a sustainable manner [10]. Because of the aforementioned, this study sets out to understand the agronomic response of two experimental varieties of habanero chili peppers identified as HNC-6 and HCC-8 to the application of band vermicomposting.

MATERIALS AND METHODS

Location of the experiment

The experiment was carried out in a macro-tunnel of the Department of Horticulture of the Universidad Autónoma Agraria Antonio Narro ($25^{\circ} 21' 23.126'' \text{ N}$ and $101^{\circ} 2' 6.801'' \text{ W}$), where the climate is dry with few rains and the temperatures range between 5° C and 24° C ; the field work was conducted in the period of May to December in 2021.

Plant material

Seeds from two experimental varieties of habanero chili pepper were used, of names HNC-6 and HCC-8, from the germplasm that is held in the Training and Center for Training and Development in Seed Technology of the Universidad Autónoma Agraria Antonio Narro.

Sowing

The experimental varieties HNC-6 and HCC-8 were sown in polystyrene trays with 200 cavities, each cavity with a mixture of peat moss and perlite substrate in a proportion of 70/30%. Application of low-volume irrigation was carried out using triple 17 (17-17-17+Me) dissolution added with microelements for the seedling nutrition, in doses of 0.5, 0.75 and 1 g L⁻¹ at the second, fourth, and sixth week after emergence, respectively, until before the transplant.

Soil preparation

In the preparation of the experimental area, first, the weeds were removed, the soil was de-compacted, and cultivation beds were formed; a canal of approximately 15 cm of depth and 20 cm of width was opened, where band vermicomposting was applied, which was integrated to the furrow; finally, the beds were reformed. The composition of the vermicompost used in this research study (applied in bands), is shown in Table 1.

Table 1. Nutritional composition of the vermicompost used in the execution of the experiment.

Element	Unit	Quantity
Total nitrogen	%	1.51
Phosphorus (P)	%	0.54
Potassium (K)	%	1.28
Calcium (Ca)	%	10.4
Magnesium (Mg)	%	0.85
Sodium (Na)	%	0.21
Sulfur(S)	%	0.37
Iron (Fe)	mg kg ⁻¹	5950
Copper (Cu)	mg kg ⁻¹	16.8
Manganese (Mn)	mg kg ⁻¹	249
Zinc (Zn)	mg kg ⁻¹	237
Boron(B)	mg kg ⁻¹	61
Humidity	%	14.4
Organic material	%	31.8
Ashes	%	68.2
Organic carbon	%	18.5
C/N ratio		12.2

Trasplanting

After the furrows were created, ribbon irrigation and padding were applied, and irrigation at field capacity; transplanting was done at double line (in herringbone pattern), with 25 cm of distance between lines and 30 cm between plants; the distance between cultivation beds was 1.5 m, and the approximate plantation density was 44,000 plants ha^{-1} .

Description of the treatments

Two experimental varieties of habanero chili peppers were evaluated, called HNC-6 and HCC-8, treated with five doses of vermicompost: 0, 0.3, 0.6, 0.9 and 1.2 kg plant^{-1} . The distribution of treatments was with a completely random design with factorial arrangement of 2×5 , with a total of ten treatments with four repetitions each, and each repetition with six plants, of which only four were used placed right at the center of the experimental unit to carry out the corresponding evaluations and quantifications; the data obtained were analyzed in the Infostat[®] statistical software.

Variables evaluated

Agronomic variables

Plant height (cm): the measurements were recorded starting at 15 days after transplanting and a measuring tape graded in centimeters was used for their quantification, while the stem diameter (mm) was also quantified at 15 days after transplant with the help of a digital Steren[®] Vernier, HER-411; both response variables were evaluated every 15 days.

Yield variables

Number of fruits per plant: the total number of fruits harvested in each plant were counted and the yield of fruit in grams per plant (g) was determined with the help of a digital Steren[®] balance, MED-080; the average weight of the fruit resulted from dividing the total weight of the fruits by the number of fruits per plant (g). The calculated yield (t ha^{-1}) resulted from multiplying the yield of each plant by the total number of plants according to the plantation density established in the experiment (44,000 plants).

Variables quantified in fruit

For the measurement of fruit length and fruit diameter (mm), fruits were collected randomly on which the length and the diameter were measured in each harvest with the help of a digital Steren[®] Vernier HER-411. To determine the percentage of total soluble solids or ($^{\circ}\text{Brix}$), the fruits were macerated, and this way cell extract was obtained which was taken to a digital Soonda[®] refractometer 0-85%. And finally, to determine the firmness of the fruit (Kg cm^{-2}), data were taken with the help of a digital Force Gauge GY-4 penetrometer.

RESULTS AND DISCUSSION

According to the analysis of variance (ANOVA $p \leq 0.05$), significant statistical differences were found between varieties (Table 2). The variety HCC-8 presented plants of larger size

Table 2. Analysis of variance and means test of growth variables, yield and components, of two experimental varieties of habanero chili pepper treated with five doses of band vermicomposting.

Variety	PH (cm)	SD (mm)	GPP (g)	NFP	AFW (g)	CY (t ha ⁻¹)
HNC-6	65.00*	14.72 a	718.74 a	129.93 a	5.52 a	31.63 a
HCC-8	90.47 a	14.60 a	363.80 b	69.55 b	5.27 a	16.01 b
ANOVA p≤	<.0001	0.945	<.0001	<.0001	0.2980	<.0001
MSD	8.66	3.69	84.06	10.26	0.39	3.69
Vermicompost (kg plant ⁻¹)						
0	83.63 a	17.50 a	594.38 a	108.66 a	5.43 a	26.16 a
0.3	82.24 a	13.79 a	519.09 a	90.16 a	5.65 a	22.84 a
0.6	79.97 a	12.81 a	565.63 a	103.66 a	5.43 a	24.89 a
0.9	73.88 a	15.00 a	532.78 a	94.97 a	5.52 a	23.44 a
1.2	68.96 a	14.21 a	494.47 a	101.25 a	4.95 a	21.76 a
ANOVA p≤	0.1785	0.5516	0.5762	0.1808	0.2158	0.5657
MSD	19.50	8.32	189.19	23.10	0.88	8.32
CV(%)	17.18	38.89	23.94	15.86	11.22	23.93
Interaction						
ANOVA p≤	0.6431	0.7679	0.996	0.200	0.4041	0.9964
MSD	32.48	13.86	305.19	38.47	1.47	13.86

Different letters in the same column differ statistically (Tukey $p \leq 0.005$). MSD=minimum significant difference, CV=coefficient of variation. PH=plant height, SD=stem diameter, GPP=grams harvested per plant, NFP=number of fruits per plant, AFW=average fruit weight, CY=calculated yield (t ha⁻¹).

in their final height (90.47 cm) compared to HNC-6 (65 cm); this is below the results found by Tapia [11] who reported an average height of 137 cm, although above those reported by Camposeco [12] in chocolate habanero peppers, both studies with exclusive mineral fertilization; therefore, it is inferred that the environmental conditions of the crop and the type of fertilization influence the agronomic behavior of the varieties. In the varieties yield in grams harvested per plant (GPP), number of fruits per plant (NFP) and yield calculated in tons per hectare (t ha⁻¹), significant differences were found between varieties, with the variety HNC-6 being superior to HCC-8 in average fruit weight, while there was no significance in stem diameter. On the other hand, the doses of vermicompost applied do not exert a significant effect in any of these response variables, and statistical differences were also not observed in the interactions. In relation to this, it has been documented that the different ways of vermicomposting determine the different physical and chemical properties, which can influence the growth and the morphology of the plants in various ways, thus impacting the final yield of a crop [15].

The differential statistical response between varieties (ANOVA $p \leq 0.05$) indicates that the variety HNC-6 presented fruits with greater length, pericarp thickness, number of locules, and fruit firmness, while the variety HCC-8 only presented fruits with greater diameter. Statistical significance was not observed in total soluble solids (Table 3). This was probably due to the genetics of the variety [21], in addition to the possible influence

of the environment [22], and its high interaction with the genotype [23]. Meanwhile, no significant statistical differences were found in the treatments with vermicompost, except for the variable fruit diameter (Table 3 and 2), which was higher in the treatment control or without vermicompost. In relation to this, a very important factor to consider with the use of vermicompost or compost is the C/N rate, which influences the balance of nutrients in the environment; a C/N rate <20 indicates a high degree of stabilization of the organic matter and a satisfactory degree of maturity [13], and this condition is necessary although not sufficient, since vermicompost has a low C/N rate, lower than 18-19; vermicomposting is faster but the excess in nitrogen is released quickly as ammonia, and this process induces loss of nitrogen rapidly from volatilization, which is a fundamental element for the growth and development of plants [18]. In addition, Márquez [18] mentions that for the process of vermicomposting to be conducted correctly, the C/P rate should be between 70 and 150, while the N/P rate should be between 5 and 20. The analyses performed on the vermicompost used in this research report a C/N rate of 12.25, the C/P rate is 34.25, and the N/P rate is 2.79, values that are far below the optimal values suggested, which probably led to the loss of nitrogen [18]. This would explain the response observed in the genotypes and with the vermicompost doses tested; this condition also modifies the microbial activity, since the lack of nitrogen alters the metabolism of carbon, decreases the levels of malate and organic acids in general, and increases the levels of starch, which affects the growth, development and yield of the crop and also the quality of fruits and

Table 3. Analysis of variance and means test of fruit quality variables, from two experimental varieties of habanero chili peppers treated with five doses of band vermicompost.

Variety	FL (mm)	FD (mm)	PT (mm)	NL	TSS (°Brix)	FF (Kg cm ⁻²)
HCN-6	51.27 a*	28.90 b	1.95 a	3.60 a	9.49 a	1.99 a
HCN-8	41.86 b	33.07 a	1.52 b	3.05 b	9.91 a	1.78 b
ANOVA≤	<.0001	<.0001	<.0001	0.0002	0.3515	0.005
MSD	1.8977	1.8063	0.0813	0.2611	0.9040	0.1425
Vermicompost (kg plant ⁻¹)						
0	46.37 a	32.89 a	1.74 a	3.25 a	8.75 a	1.72 a
0.3	48.52 a	31.10 ab	1.73 a	3.25 a	9.73 a	1.88 a
0.6	46.39 a	31.44 ab	1.77 a	3.50 a	9.79 a	1.99 a
0.9	46.32 a	29.85 b	1.77 a	3.25 a	9.56 a	1.89 a
1.2	45.23 a	29.67 b	1.65 a	3.38 a	10.68 a	1.96 a
ANOVA≤	0.2806	0.0040	0.3417	0.6541	0.1334	0.1479
MSD	4.27	2.43	0.18	0.58	2.03	0.32
CV (%)	6.28	5.37	7.24	12.11	14.36	11.63
Interaction						
ANOVA≤	0.033	0.0177	0.2050	0.9594	0.0117	0.94
MSD	7.11	4.05	0.30	0.97	3.38	0.53

Different letters in the same column differ statistically (Tukey $p \leq 0.005$). MSD=minimum significant difference, CV=coefficient of variation. FL=fruit length, FD=fruit diameter, PT=pericarp thickness, NL=number of locules, TSS=total soluble solids, FF=fruit firmness.

seeds; in addition, it alters the content of vitamins, sugar and soluble solids [19, 20], which also causes an inadequate proliferation of microbial fauna from the lack of phosphorus available. Therefore, it is inferred that what was described before, together with the lack of maturity of the vermicompost used, did not allow observing differences between the doses used in this experiment.

No significant statistical differences were found between the vermicompost doses applied for the variable plant height (ANOVA $p \leq 0.05$); however, a trend is seen in the growth of both varieties through time, where at the end of the cycle, the applications of 0.3 and 0.6 kilograms of vermicompost per plant resulted in slightly larger plants compared to the control (Figure 1). The main element present in organic matter is carbon, fundamental for the photosynthetic process [13], and it has nutritional functions in the plants due to its action in the generation of carbohydrates together with solar radiation and water,

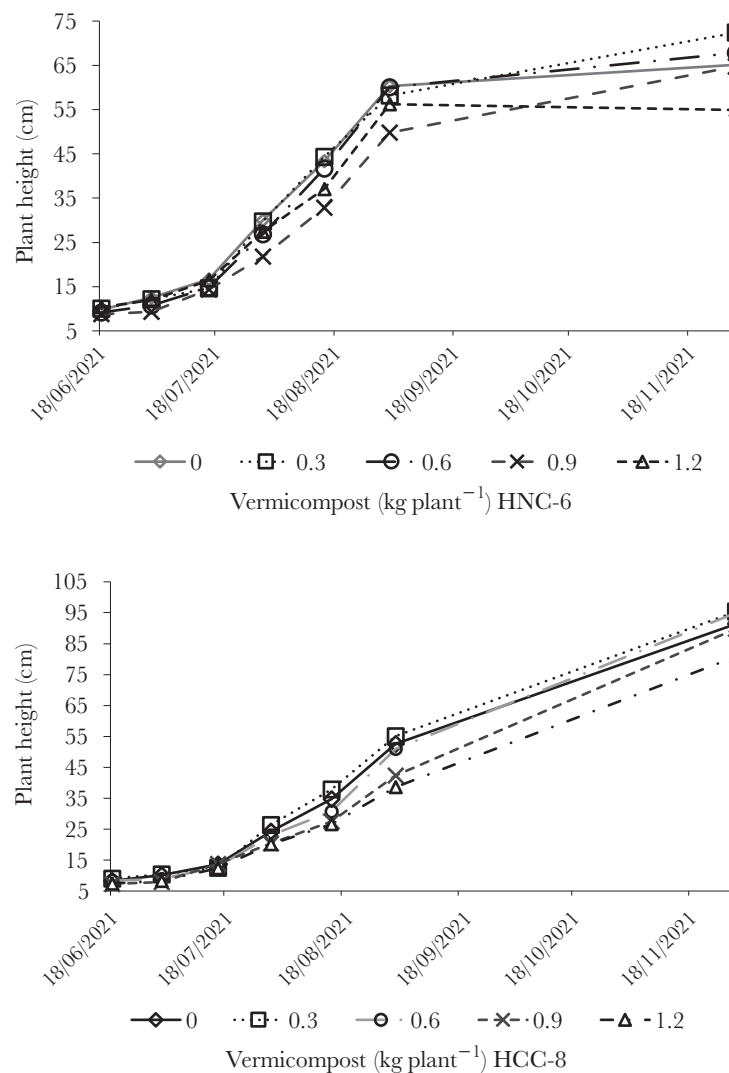


Figure 1. Growth curve of plant height in varieties HNC-6 and HCC-8 with the application of different doses of band vermicompost.

and in the catabolism of microorganisms that happens in organic compounds [14]. This is because with higher amounts of organic carbon, the soil pH improves, the apparent density decreases, the porosity and aeration capacity improve, the water retention capacity increases, and there is a higher microbial population in the soil, in addition to increasing the soil fertility, favoring nutrient absorption and consequently the growth, development and yield of crops [15,16].

In the case of stem diameter, no significant differences were found between varieties; however, when observing the growth of stem diameter in both varieties, the vermicompost dose of 0.9 kg results in thicker stems at the end of the cycle, followed by the control (Figure 2). With vermicompost applied as soil improver in some crops such as pepper, tomato and strawberry, it has been discovered that it is a source of macro and micronutrients, biologically active metabolites as growth regulators, humates, vitamins, enzymes, antibiotics and the presence of microorganisms that mainly improve the biological fixation of nitrogen and the solubilization of phosphorus [13, 15, 17].

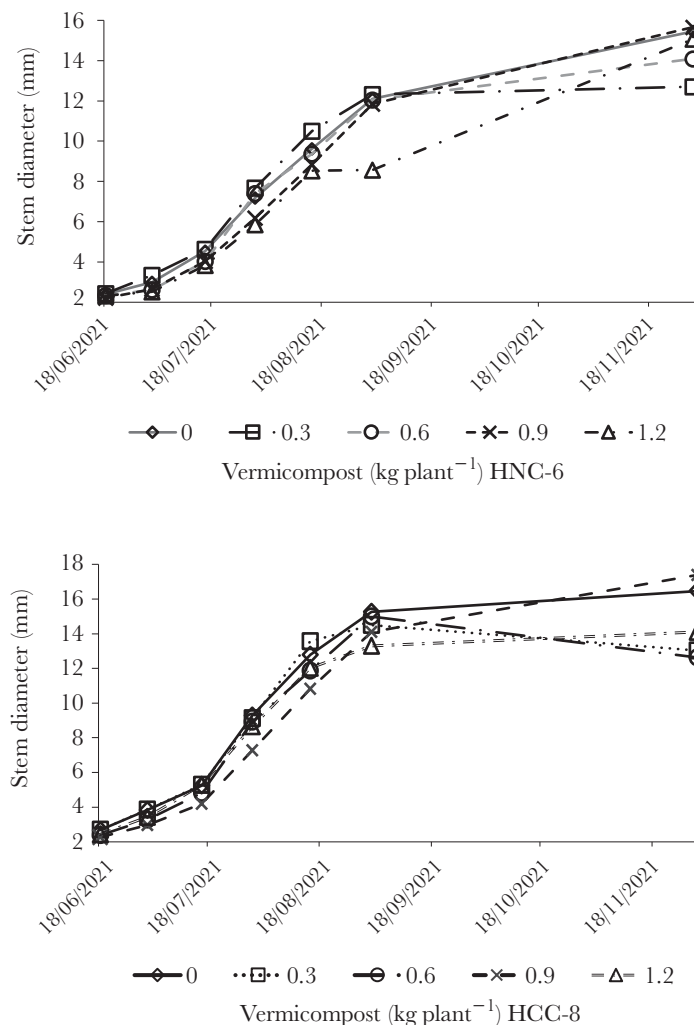


Figure 2. Growth curve of stem diameter of the varieties HNC-6 and HCC-8 with the application of different doses of band vermicompost.

Significant differences were present in the interactions of the variables fruit length, fruit diameter and TSS; in the variables length and diameter of fruit, the variety HNC-6 presented longer fruits when 0.3 kg of vermicompost was applied per plant, while the fruits with greater length and diameter in the variety HCC-8 were found with the control treatment and with 0.3 kg of vermicompost per plant. In relation to this, there are studies that indicate that the size of the orange habanero pepper grown in the greenhouse reaches on average 3.58 cm of length and 2.47 cm of diameter [24], the fruits of variety HNC-6 are larger with 5.12 and 2.89 cm, respectively. In the case of chocolate habanero peppers, fruits with 2.92 cm of length and 2.44 cm of diameter were reported [11], while the fruits of the variety HCC-8 are also chocolate color, with 4.18 and 3.3 cm, respectively, are above these values.

Authors such as De Avila [25] observed different behaviors in different cultivars of *C. chinense* in terms of lanyard and size of the fruits, and pointed to the lack of nitrogen decreasing the biomass and favoring the accumulation of starch in the leaves, which could be the reason why the variety HCC-8 produced fruits with smaller diameters in

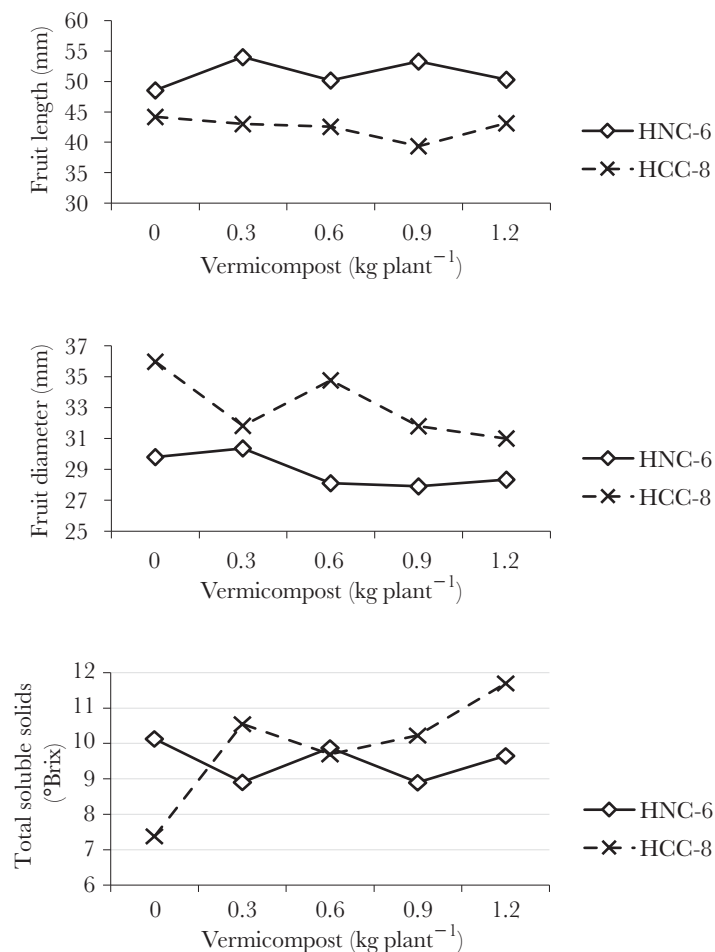


Figure 3. Significant interactions observed between variables HNC-6 and HCC-8, under the application of different doses of band vermicompost.

the vermicompost treatments, since the plants present lower fruit setting but of larger size when the levels of ammonium increase in the soil solution, as a tolerance mechanism [20].

For the case of total soluble solids (°Brix), a higher content was found in fruits of the variety HCC-8, and an increasing trend was seen in total soluble solids as the dose of vermicompost increased. In the case of the HNC-6 variety, it presented lower content of soluble solids than HCC-8, and in this sense, it has been reported that the content of total soluble solids is related to the taste of the fruits and the accumulation of sugars by the plant, and which are generally accumulated in the fruits [26]; these compounds increase when the fruit matures, because they are the main source of energy for respiration [27].

CONCLUSIONS

The experimental variety HNC-6 showed a better agronomic response under the conditions tested compared to HCC-8. The vermicompost doses exerted a similar response and trend effect in the two experimental varieties of habanero chili peppers tested.

In the interaction of the varieties and the vermicompost doses, significant differences were present for the variables FL, FD and TSS, and therefore, the application of vermicompost only influenced some aspects of the fruit quality.

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Soy (*Glycine max* L.) production and importation determinants in Mexico and actions aimed to increase its domestic coverage

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ABSTRACT

Objective: To analyze soy (*Glycine max* L.) production and importation determinants in Mexico, during the 1990-2000 period.

Design/Methodology/Approach: Using lineal models, two econometric models were developed, in order to evaluate the relation between the soy production, harvested area, and soy production vs. importation variables.

Results: We confirmed that soy production in Mexico is related to the increase of the harvested area, which promotes an increase in the domestic market and a reorganization of the soy harvested areas. The second model verified that the importations are the result of the limited domestic production. We propose a set of actions to increase domestic soy production.

Study Limitations/Implications: The models must consider new variables to broaden the determinants and to improve the public policy actions proposed.

Findings/Conclusions: The low domestic coverage rate and the high dependency on imports requires the implementation of policy actions to improve the domestic production capacity. A specific set of actions is proposed.

Keywords: soy production, soy importation, harvested area, econometric models, public policy actions.

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INTRODUCTION

Soy (*Glycine max* L.) is one of the most dynamic food markets in the world, as a result of the growth of the production, the consumption, the cultivation areas, and the exportation. Between 1990 and 2020, the soy cultivation area worldwide increased 121% (about 70 million hectares) and the production volume multiplied by 3.1%, reaching 336 million tons. In 1990, a ton of soy costed US\$357. The prices increased since the beginning of the new century, reaching US\$670 in 2012 (World Bank, 2018). Recently, prices have escalated again. This price escalation has increased during the last



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months, as part of the inflationary process that has impacted all food prices around the world. Specifically, the price of soy increased 105% between May 2020 and June 2022 (FAOSTAT, 2022).

Soy is a high value input for the vegetable oil industry and the biodiesel production industry. This oilseed has a wide range of uses, as a consequence of its high protein and oil content. Dried soy seeds are made up of 20% oil and 40% protein. Researches carried out by the food industry have led to the incorporation of soy into a wide variety of foods, both for human and animal nutrition. The American Soybean Association (ASA) has been a very effective pressure tool used by farmers to develop a consumption market for American soy. In Mexico, it has supported or developed all sort of actions to promote soy demand (García-Fernández *et al.*, 2018).

In terms of volume, soy production in Mexico has been stagnant since the late 1980s. Those levels have not been surpassed yet. The highest production volume (700 thousand tons) was reached from 1985 to 1989 (García Fernández *et al.*, 2018). Between 1990 and 1994, soy production remained at high levels, reaching its maximum point in 1991. In 1995—the year that Mexico entered the North American Free Trade Agreement (NAFTA)—, a contraction process began, both in production and in area. Compared with the previous year, soy production and harvested areas diminished 63% and 53%, respectively (Claridades Agropecuarias, 1997). The domestic market continued to expand: between 1980 and 2020, the apparent consumption increased by 225%. The maximum coverage (40%) of the domestic market was reached in 1990. Mexico experienced a fall in domestic production, harvested areas, and domestic coverage. Consequently, a 592% increase in importations was recorded during that same period (SIAP, 2022). Between 2010 and 2020, the domestic soy market increased by 30% (apparent consumption). However, the capacity of Mexican soy to supply the market is very limited, accounting for only 4% (Table 1).

Specifically, this study seeks to identify the soy production and importation determinants in Mexico, using two econometric models in order to establish the soy production and importation behavior in the country. The two models showed that the changes in soy production were mainly the consequence of the increase of the harvested area and the

Table 1. Production, imports, exports, harvested area, and self-consumption of soy in Mexico (selected years: 1990-2022).

Year	Production (Q) ton.	Yield (Ton/Ha)	Harvested Area (AC) (ha)	Exports (X) ton	Imports (M) ton	Apparent Consumption (C)(ton). $C=Q+M-X$	Self-consumption (Q/C)
1990	575,366	2.01	285,615	74	897,021	1,472,313	0.39
1991	724,969	2.12	341,679	0	1,489,310	2,214,279	0.33
2000	102,314	1.46	69,969	1,728	3,984,886	4,085,472	0.03
2006	81,113	1.50	54,211	191	3,765,610	3,846,532	0.02
2017	432,927	1.65	262,602	100	4,343,000	4,775,827	0.09
2018	324,210	1.70	190,628	0	4,343,000	4,667,210	0.07
2019	232,679	1.59	145,923	0	4,853,000	5,085,679	0.05
2020	246,019	1.57	156,979	0	5,727,000	5,973,019	0.04

Source: SIAP, 2022.

importation. This increase is the result of the soy production contraction, although other variables also influenced the results. Based on these results, a set of public policy actions are proposed to increase domestic soy production.

MATERIALS AND METHODS

This research sought to establish the relationship between the *Producción* (*Production*) dependent variable and the *Superficie cosechada* (*Harvested Area*) and *Importaciones* (*Imports*) independent variables, using the stepwise method. First, a search for a relationship between each independent variable and *Producción* was carried out, including only the significant variables. Subsequently, a multiple regression model was tested—returning to a simple lineal regression model, if the determination coefficient did not significantly improve. R (R Core Team, 2022) was used to perform all the statistical analysis and the *lmtest* package software was used to diagnose the models (Achim and Hothorn, 2002).

Two econometric models were tested based on the objectives of this study, in order to identify the variables that impacted the behavior of soy production and importation in Mexico, during the 1990-2020 period. The two models evaluated the relationship between soy production, soy importation, and the harvested area in the country. In the mid-1990s (1994), soy production in Mexico grew, reaching its maximum point in 1991. This maximum point has not been surpassed yet. Soy production diminished and soy importation increased in order to cover the growing domestic market, after the NAFTA and the agricultural liberalization measures entered into force.

The two econometric models attempted to explain the domestic soy production, based on the area variable (as dependent variable), during the 1990-2020 period. A lineal regression was developed to understand the harvested area effect in soy production. The second model attempted to explain the relationship between soy importation and soy production, as a dependent variable.

RESULTS AND DISCUSSION

The results of the two developed and tested econometric models are included below. Subsequently, the results are discussed. On the one hand, the fall and the scarce soy production capacity in Mexico is explained. On the other hand, a set of actions is proposed to increase domestic soy production.

The following equation was used for the first model:

$$y_{ij} = \beta_0 + \beta_1 x_j + \varepsilon_{ij} \quad (1)$$

Where: y_{ij} is *Producción_i* (*Production_i*) in soy tons in *Superficie_j* (*Area_j*). x_j is *Superficie_j* harvested in hectares. ε_{ij} is the random error of *Producción_i* in *Superficie_j*.

Assumption of the model:

$$y_{ij} \sim \text{NI}(\mu_y, \sigma_y^2) \quad (2)$$

$$x_j \sim \text{NI}(\mu_x, \sigma_x^2) \quad (3)$$

$$\varepsilon_{ij} \sim \text{NI}(0, \sigma^2) \quad (4)$$

$$V(\varepsilon_{ij}) = \sigma^2 \quad (5)$$

Hypothesis:

$$H_0 : \beta_1 = 0$$

$$H_1 : \beta_1 \neq 0$$

A lineal regression was carried out in order to understand the harvested area effect on soy production. To evaluate linearity between variables, a scatter plot was developed, along with the line of the estimated lineal regression model (Figure 1). The visual examination indicated a lineal relationship between harvested area and production. The visual examination of histograms and graphs of the standardized residuals suggests normality; however, there was no homoscedasticity. Consequently, several models were evaluated, using the logarithmic transformation of the production and the harvested area, one at the time, and both at the same time.

The model that best corrected the homoscedasticity problems was obtained using a logarithmic transformation of *Producción*. The visual examination of the graphs and

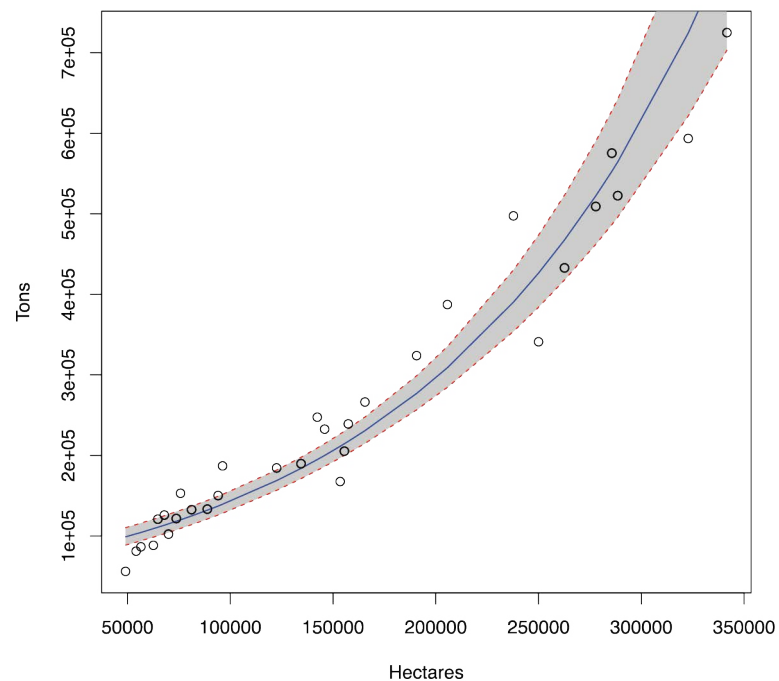


Figure 1. Relationship between soy production and harvested area during the 1990-2020 period. The figure was developed by the authors.

the application of both the Shapiro-Wilks (0.941, $p=0.08819$) and the Kolmogorov-Smirnov (0.1077, $p=0.8872$) tests showed that the standardized residuals had a normal distribution. Meanwhile, the Breusch-Pagan ($\chi^2(1)=1.624$, $p=0.2025$) test confirmed the homoscedasticity of the errors of the variance.

The following prediction equation was used:

$$Producción_i = \exp^{11.144771+7.275 \times 10^{-6}} (Superficie_j)$$

The soy production was predicted in a statistically significant way, based on the harvested area $F(1, 29)=339.1$, $p<.001$, counting 92% of the production variation with a R^2 adjusted=91.85%.

The second model tried to explain the relationship between the soy importation and production. When NAFTA came into force in 1994, the soy production and soy importation trends in the country changed. Soy importation grew at very high rates, while soy production has remained stagnant.

Another evaluated econometric model links *Importaciones (Importation)* as a dependent variable of the *Producción (Production)*, *Superficie (Area)*, and *Precio (Price)* as independent variables. *Producción* was the only independent significant variable that can determine the behavior of importation during the analyzed period.

The equation of the second model was as follows:

$$y_{ij} = \beta_0 + \beta_1 x_j + \varepsilon_{ij} \quad (6)$$

Where: y_{ij} are the *Importaciones_i (Importation_i)* in US dollars, along with the domestic *Producción_j (Production_j)*. x_j is domestic soy *Producción_j (Production_j)* in tons. ε_{ij} is the random error of *Importaciones_i*, with *Production_j*.

Assumption of the model:

$$y_{ij} \sim \text{NI}(\mu_y, \sigma_y^2) \quad (7)$$

$$x_j \sim \text{NI}(\mu_x, \sigma_x^2) \quad (8)$$

$$\varepsilon_{ij} \sim \text{NI}(0, \sigma^2) \quad (9)$$

Hypothesis:

$$H_0 : \beta_1 = 0$$

$$H_1 : \beta_1 \neq 0$$

In order to evaluate the linearity between variables, a scatter plot was developed, along with the line of the estimated lineal regression model. The visual examination indicated a

lineal relationship between importation and production (Figure 2). The visual examination of the histograms and graphs, as well as application of the Shapiro-Wilks (0.9636, $p=0.3819$) and Kolmogorov-Smirnov (0.136, $p=0.5887$) tests, showed that the standardized residuals had a normal distribution. Meanwhile, the Breusch-Pagan ($\chi^2(1) = 3.321, p = 0.06842$) test confirmed the homoscedasticity.

The prediction equation was the following:

$$\text{Importaciones}_i = 4243144 - 2.89 \text{ Producción}_j$$

which means that a reduction of 2.89 tons of imports is expected per ton of soy production. Soy importation was predicted in a statistically significant way by production, $F(1, 29) = 12.44, p = 0.001$, with 30% variation in importation.

Relationship between production, importation, and harvested area

The two models confirmed the effects of the production, harvested area, and importation variables on soy production and importation in Mexico. During the 1990-2020 period, the increase of the domestic soy production resulted from changes in the harvested areas and not from technological efficiencies. Consequently, the modest increases of the period were mainly the result ($R^2 = 92\%$) of an expansion of the sowing area. This situation proves that this factor is the main source of growth. Derived from the possible increase in productivity, yield and other factors were not the cause of this increase. However, this factor alone is not enough to guarantee a soy production increase in the country. The increase of the area is

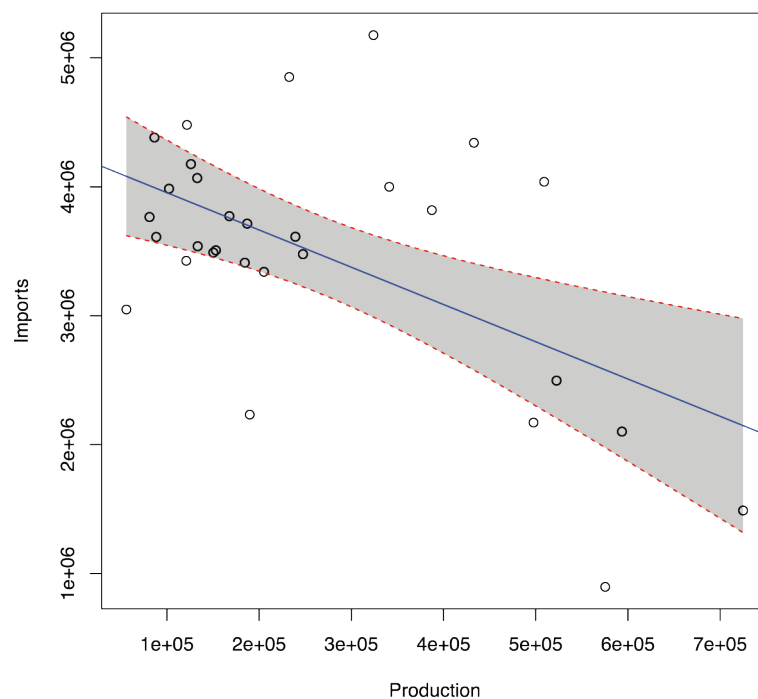


Figure 2. Relationship between importation and production during the 1990-2020 period. Figure developed by the authors.

a limited factor, because soy competes with other products that can also attract productive investment.

The second model proved that imports are directly determined by the production variable. This result shows that the increase in the domestic market was mostly met using imports, given the low sensitivity of the domestic offer to respond to the stimulus of the demand. This phenomenon can be explained by a R^2 of 30%, which means that other factors are involved in the importation level, but were not taken into consideration in the model. These factors led to an exponential growth of imports during the said period.

An approach to the limited domestic production

Between 2000 and 2020, the commercialized value, the volume, and the harvested area grew at an average annual rate of 15%, 6.44%, and 4.73%, respectively (SIAP, 2022). Yields have not grown; quite the opposite, they remained stagnant, although there were important differences among states. The domestic average was 1.35 t ha^{-1} —lower than the average value in the northwestern Mexico and far below the yields of the leading countries.

Several factors are responsible for the low yields of the domestic production. One factor is the cost of technology, machinery, fertilizers, and technological packages in the domestic market, which are higher than the relative prices that producers pay in leading countries. Another factor that impacts soy production is the productive model. After the Mexican Revolution, the land was divided into plots. These small properties (*ejidos*) are still the dominant type of private property in the country. Meanwhile, in the main world producers (the United States, Brazil, and Argentina) ranchers own large properties. The situation in Mexico is very different, since the smallholding structure still prevails (Escobar, 2016; Urioste, 2012). This difference mainly impacts the productivity of certain crops, such as grains, which require machinery and technology to appropriate economies of scale, which are a source of high productivity for this type of crop. Other crops require a lower scale to achieve high productivity and, therefore, their technification level is lower. From the mid-1980s, the implementation of liberalization policies in the Mexican economy started to rupture the ejido structure. In 2013, 77% of the land structure was still made up of <5.0 -ha *ejidos* (SAGARPA, 2013). Consequently, the size of the agricultural exploitation areas has been a key limitation for the spread of the technological packages of the multinational companies (García Fernández *et al.*, 2014).

Actions proposed to increase the domestic soy production

As a consequence of the permanent soy deficit of the country, importation reaches an annual expenditure of approximately US\$3,000,000. After corn, soy is the most important grain included among the Mexican agricultural imports. Several actions related to the soy value chain are required to strengthen strategies that should be carried out by the private sector and the different government institutions, in order to promote an increase in the domestic production.

One of the main actions is the urgency to provide affordable interest rates for rural producers and companies that require fundings. Currently, the inequality regarding foreign competitors is huge. Several private and public banks offer this kind of fundings, but their

offers are not attractive enough to make the risk worthwhile. In addition, the bureaucratic costs (mainly the waste of time) discourage this type of procedure. The fundings must not only take into consideration the production elements, but also include the purchase market risk management tools, which can help producers to mitigate in advance their uncertainty.

Currently, producers cannot afford purchasing new machinery, as a consequence of the low profitability levels. Therefore, productive arrangements for collective groups (productive and commercialization associations) are a feasible alternative when managed as a company. At this point, the public sector can play an important role, providing the tools required to encourage the purchase of technology and materials. The federal government can also encourage the creation of producer associations, awarding various prizes, tax reductions, or another type of incentives.

At regional level, Mexico has experts on soy cultivation. Consequently, the Mexican government must actively promote knowledge transference. The experts can help the producers depending on the specific needs of their production units, adapting technological packages and optimizing the resources to reduce production costs.

CONCLUSIONS

Increasing the consumption of soy in Mexico is a major pending issue. Despite the progressive increase of the demand, soy production has remained stagnant and has not surpassed the levels reached before the 1990s. The prevailing productive model in Mexico accounts for the setback of the domestic production, which impacts soy and all grains. The situation became more pronounced once the NAFTA came into force. The agreement promoted and deepened the specialization of the country's economy in certain goods and increased the dependency on other imports (*e.g.*, almost all grains). This overview of the economy of the country—regarding the specialization of the agricultural sector—forces the sector, as part of a development strategy, to implement a set of actions, aimed to the improvement of food safety in the domestic grain production.

The econometric models verified the role of the increase of the harvested area in the evolution of the domestic soy production. The predominant productive model is based on the extensive growth of the land productive factor. Although some actions have been implemented to promote the use of science and technology as a growth factor, the efforts have not managed to promote a change in the productive model and to respond to the growth of the demand through an intensive growth of the offer. There is still a long way to grow at the pace that the domestic demand requires. Therefore, the second econometric model proved that soy importation has been determined by the scarce capacity of the domestic production to meet the demands of the growing domestic market.

The proposed actions seek to provide affordable fundings for producers, to encourage them to create associations, to award productivity prizes, and to increase resources for the research about oilseeds and the transference activities with producers. All these actions should modify the grain productive model in the country and could contribute to the transformation of the Mexican agricultural sector, and the life conditions of producers, particularly medium and small producers.

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Perception of inhabitants from the Laguna Santiaguillo Basin: natural resources and livelihoods

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ABSTRACT

Objective: To learn about the state of the natural resources and productive systems in the Laguna Santiaguillo Basin from the viewpoint of the inhabitants.

Methodology: The approach was qualitative, by means of a focus group.

Results: The problems perceived are related to: access to water both because of its low availability and due to the inequity in its distribution which generates low productivity and conflicts over the use of this resource; low prices for agricultural products; conflicts arising from the use of wildlife; and the degradation of grasslands as a result of overgrazing.

Limitations on study/implications: The Mennonite group, which is an important actor within the basin, was not represented in the focus group.

Conclusions: The participants in the group perceive the basin's problem in an integrated manner, linking components of natural, economic, social and political resources, which lead them to actions adapted to their context to solve this problem.

Keywords: Socioenvironmental conflicts, perception, focus group, ecosystem degradation, endorheic basin.

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INTRODUCTION

In the management of landscapes, natural resources and territories, the inhabitants' perception is an important element both at the individual and the collective level; it is strongly influenced by the community's culture, context and historical processes, which result in an appropriation and management of natural resources derived from the perceptual references of each region (López-Zapata *et al.* 2018). When perceiving, not only is the information from the senses integrated, but in this process, the knowledge, experiences and ideas of the observer also intervene, among other cultural factors that produce knowledge or experiences about the environment (Durann 2009; García *et al.*, 2018; Rodríguez-Rodríguez *et al.* 2021).

Presently, the perception of natural resources is an issue that has acquired greater relevance (Amin *et al.*, 2015; Frank *et al.*, 2017; Baffoe and Matsuda 2018) because the way in which resources are used for the satisfaction of needs and the procurement of livelihoods depends to a large extent on the perception, and as a result of pressures that they suffer or which have been aggravated, among other things, by climate change and the lack of sustainable practices that allow their conservation (Orellana Salas and Lalvay Portilla 2018). Therefore, the diversity of ideas, cultures, appropriations and thoughts which lead to perception become essential to agree on actions to revert situations of degradation of the territory and the productive systems (Courage and Saarinen, 2020).

The Laguna Santiaguillo Basin, located in Durango, Mexico, is presented as case study. The main economic activities of the primary sector are irrigation and rainfed agriculture, extensive livestock production and forestry (SRNyMA, 2018). However, some of the lands devoted to these activities suffer deterioration due to an inadequate historical management. The basin is endorheic and within it there is a wetland of international importance designated in 2012 as a Ramsar 2046 site (SEMARNAT, 2021). In this study, the status of the natural resources was analyzed through the inhabitants' perception about them, supported by studies that have been conducted in the basin, which will allow establishing strategies for its sustainable management and conservation.

MATERIALS AND METHODS

A focus group was established as a technique through group discussion, with the aim of identifying the feelings, thoughts and experiences of participants in relation to the theme of interest, and of obtaining qualitative data (Mena and Méndez 2009). The group was held in October, 2018, with inhabitants from the different regions of the basin represented by the so-called Active Committee of the Santiaguillo Basin, whose mission is to generate mechanisms for environmental governance and to unleash local development processes (Cassio and Sánchez 2017).

Twenty-three members of the Committee attended the group, as well as four service providers and five trainers. The profile of the participants was the following: agriculture, livestock or forestry producers who live and have their productive activities within the basin; the service providers carry out their activities within the basin. An image of the basin on a blackboard was used to understand the participants' perception regarding their resources and problems, and the main types of soil coverage in the basin around it, which are: forest (including pine, pine-oak and oak forests), grasslands, agricultural fields, scrubland, lagoon and towns. Relation lines were established between each type of coverage, and finally there were questions about the perception of the status of each of the territorial elements (resources or spaces), with the response categories being: the same as 10 years ago, decreased since 10 years ago, and increased since 10 years ago. In a third phase, the assistants were grouped into work tables from each sector (agriculture, livestock production, forestry, towns and lagoon) to see the perception of the problems of each with greater detail.

Study area. The Laguna Santiaguillo Basin is located in the center of the state of Durango, Mexico, in the municipalities of Nuevo Ideal, Santiago Papasquiario, Canatlán,

San Juan del Río, Coneto de Comonfort and Santa María del Oro, of which the first concentrates most of the populations and productive activities that are developed in the basin. The basin has a surface of 254,000 hectares and it is endorheic (Figure 1). Laguna Santiaguillo has been declared a RAMSAR wetland with the label 2046 due to the importance it has for migratory birds, especially goose, ducks and cranes that spend the winter in this body of water, and from this that its management, protection and conservation has international relevance (RAMSAR 2012).

RESULTS AND DISCUSSION

The results from the discussion group are shown in Figure 2, which shows the existing relationships between the territorial components, the products, and the problems perceived in the focus. The territorial resources are shown in brown, the products that are obtained from each in green, the aquifer in blue, and the perceived problems in purple. In the same way, the arrows indicate the resources which, in agreement with the participants' perception, increased, decreased or maintained their quality or surface.

The agreements on perceptions of each of the territorial resources are presented in Table 1. In it, the consensual contributions on the problems, attributions and characteristics of each of the territorial resources can be observed.

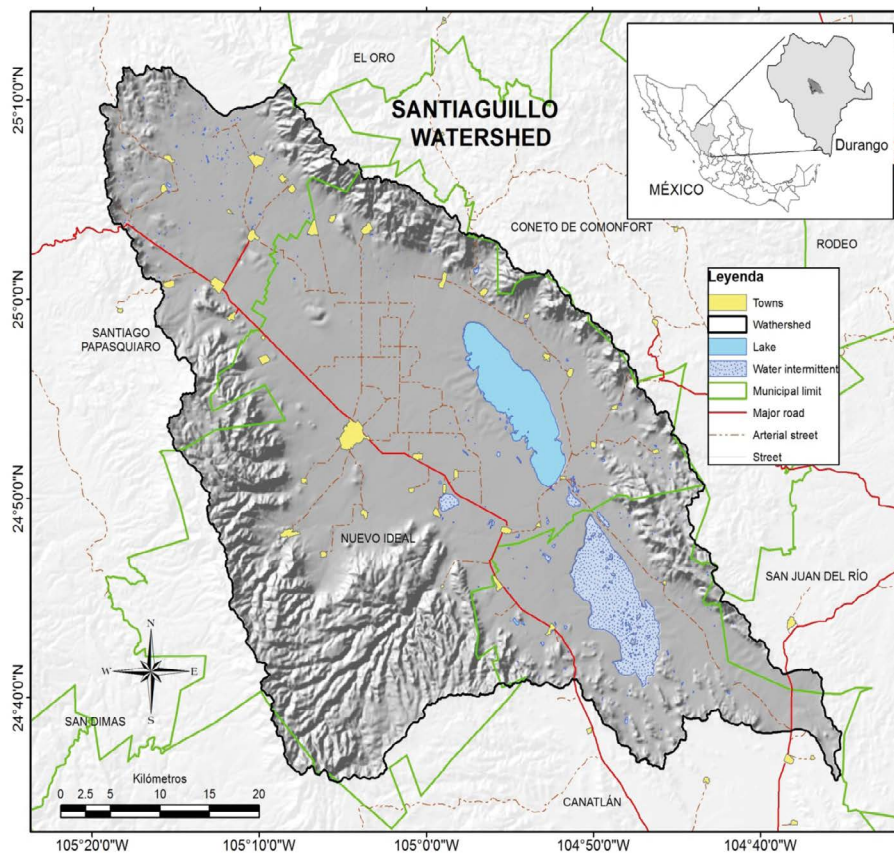


Figure 1. Location of the study area. Prepared by the authors with data from INEGI.

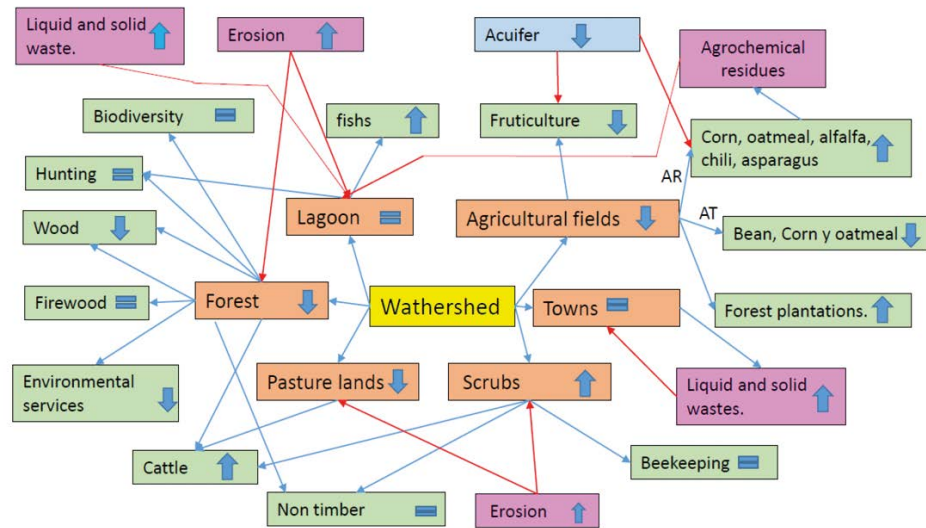


Figure 2. Conceptual diagram of the relationships between the soil coverage, the products obtained based on the perceptions of participants from the discussion group, the signs indicate if the status of the resource remains the same (⇌), has increased (↑) or has decreased (↓). (AR: Irrigation agriculture; AT: Rainfed agriculture). Source: Prepared by the authors.

According to Table 1, the water problem is of greatest relevance to them, since agriculture and livestock production depend on this resource, and because it is a limiting factor, it causes conflicts with the authority since the opening of more wells is restricted by the condition of the overexploitation of the aquifer and due to an agreement published in the *Diario Oficial de la Federación* from April 5, 2013, which provisionally prohibits the perforation of new wells. Likewise, there are conflicts with the Mennonite population over water since they have clandestine wells or some that are not regulated by the authority, as a result of the Mexican agreement signed in 1921 by which it is established that this group is not subject to the regulations imposed by Mexican Laws (Hansen 2005).

The participants of the focus group perceive that water from the aquifer ought to be extracted from deeper (Table 1), which agrees with the studies carried out by Corral-Bermúdez *et al.* (2019), who reported a negative water balance. However, as was mentioned before, this is an issue of social, productive and political conflict due to the need of all the rural actors involved for this essential resource used in every productive activity. The water balance indicates that only two of the 11 micro-basins have a positive balance, and most have catastrophically low balances (Corral-Bermúdez *et al.*, 2019a), which is why it is necessary and urgent to modernize its use.

When it comes to rainfed agriculture, participants indicated that there are low yields due to the lack of rain and the high cost of inputs which have led to ceasing to farm land and with that to the decrease of surface planted year after year (Table 1). However, as it happens in other places of the state of Durango and the basin, this activity continues to be conducted because it is a traditional way of life and of peasant identity for the farmers, particularly the older ones (Morales-De Casas *et al.*, 2021). The participants also mention that supports or subsidies given by the government are insufficient to make this activity

Table 1. Participants' perception of the focus groups with regards to the status or condition of the territorial resources of the Santiaguillo Basin.

RESOURCES	PARTICIPANTS' PERCEPTION
RAINFED AGRICULTURE	It has decreased in area because it is not very profitable. It has decreased in area because it is not very profitable.
	Corn, beans, oats and apple orchards are planted.
	Most of the apple trees are old and of low productivity.
	Lack of rainwater does not allow to ensure harvests, so groundwater is required to reduce this risk factor.
	The cost of fertilizers and herbicides is very high, so they are hardly used.
	SAGARPA and SAGDR support is insufficient. SAGARPA and SAGDR support is insufficient.
	Some rainfed agricultural fields changed their use to make plantations of <i>Pinus greggii</i> although it is necessary to wait at least 15 years to harvest and obtain some income.
IRRIGATED AGRICULTURE	Corn, oats, alfalfa, chili and, on a very small scale, asparagus are planted.
	More and more land is being planted with clandestine wells.
	There are no permits to extract more water legally.
	Mennonite Group has greater facilities to extract water legally.
	Everyone wants more water for agriculture.
PASTIZALES	They have decreased in quality and quantity
	Erosion is present
	There is an invasion of inedible plants for livestock due to people putting more and more cattle in the pastures to maintain production.
	The Secretaría de Agricultura, Ganadería y Desarrollo Rural (SAGDR) only intervenes to regulate the sale of livestock in terms of health.
MATORRALS	Have increased in surface area and are subject to erosion
	They obtain firewood, pasture for cattle and some people have apiaries.
	Low production due to increase of non-edible plants
	Erosion is a consequence of the increase in livestock, as there is no regulation of the number of cattle entering these fields.
	There has been support for erosion control from CONAFOR.
FOREST	They have decreased in surface area.
	Timber is extracted and in some ejidos there is hunting of wild turkeys and wild boars.
	They provide environmental services..
	They have erosion problems.
	There are ejidos with forest management programs.
SANTIAGUILLO LAGOON	It remains the same as it was ten years ago.
	Fish are caught in the northern part.
	Annual and temporal variations,
	Migratory birds continue to arrive at the lagoon.
	Sediment on the banks when the water level drops.
	Mennonites and villagers use the lagoon for picnics and celebrations.
	Problems with hunters leaving gates open and cattle leaving the grazing fields
AQUIFER	Year after year, water is obtained at greater depths.
	The authority does not allow any more wells to be drilled.
	There are many illegal wells.
	Mennonites have many wells and it is believed that they are not legal.
	More wells are required to increase production.
CYNEGETIC USE	This is done through the Management Units for the Conservation and Use of Wildlife (UMAS).
	The UMAS have leases with hunting organizers from the states of Jalisco and Monterrey.
	There is a lack of local regulation of UMAs
	There are few or no benefits from these activities for local residents.
TOWNS	Hunters are not responsible for crop damage, litter left behind and opening of fences.
	The population has decreased due to migration to the USA.
	There are no public services such as sewage and garbage collection.
	There are clandestine dumps near the villages that cause bad odors, flies and rats.
	Cattle die from ingestion of plastics.

profitable and, instead, they work as a subsidy to the family economy rather than to increase agricultural productivity.

Concerning the grasslands and scrublands in the basin, the consensus was focused on the decrease in the quality of the rangelands due to overgrazing because the load capacity is constantly exceeded, which results in a lower weight and even mortality of the animals (Table 1). Overgrazing is a factor that unleashes various processes which decrease the quality of the pastureland and provoke erosion (De Villalobos 2013). For the inhabitants of the basin this is particularly important since the livestock represents an important source of income for their family economy. It was also exposed that government agencies do not regulate the number of heads within the rangelands, which is a factor that favors overgrazing and with that the increase in erosion and they only focus on the livestock's health for their sale.

Regarding the forests, the perception is that they are important for the production of timber, firewood and other environmental services, and that they present erosion problems and a reduction in the forest limit due to the increase in areas used for livestock production (Table 1). However, given that most of the forest zones have forestry management programs authorized by SEMARNAT, and backing from CONAFOR, this resource is not perceived as a serious problem.

When it comes to the use for hunting (Table 1), the participants' perception indicates that the benefits derived from this activity do not reach the *ejido* owners, but rather that they remain with the hunt organizers, in addition to them not assuming the responsibility for damages produced in the fields where hunting takes place. This is a cause for dissatisfaction since the owners of the concessions are unknown, or where the benefits of this activity remain. Participants perceive the need to regulate the way of operating Wildlife Management Units (*Unidades de Manejo de Vida Silvestre*, UMAs), since according to what is established in the General Wildlife Law, monitoring the hunt should be done through reports about the use of the UMAs which must be presented annually and shared with the inhabitants.

Because of this, the members of the focus group perceive clearly the problems that there are in the basin, which agree to a greater or lesser extent with the information generated by disciplinary studies. However, the problems that are perceived continue to worsen (Figure 2), due to the lack of institutional presence, on the one hand, as is the case of water distribution and degradation of rangelands, but also because of external problems such as the low prices of agricultural products and the dependency on rainfall, which generates low productivity of the rainfed lands. On the other hand, the fact that the perception of the problem is clearer has given rise to local initiatives through the "Active Committee of the Santiaguillo Basin", which has carried out various actions since its formation with different institutions such as PRONATURA and DUMAC; however, this requires not only of the committee's activity, but also of the government institutions that support politically and economically the initiatives of the population to improve their living conditions, at the same time that they improve and increase their natural capital.

Thus, the focus group's participants perceive the quandary of their natural resources and how these problems affect the productive systems, which at the same time has a

direct effect on their economy. As was mentioned at the start of this study, perception is an element of utmost importance to establish mechanisms for governance and local development, which lead to assertive actions for the sustainable management of the basin.

CONCLUSIONS

According to the results obtained from the focus group, the participants perceive and recognize the quandary of the natural resources of Laguna Santiaguillo Basin related to their livelihoods, as well as the economic, social and political aspects that are a hindrance for their solution, for which it is necessary to generate strategies for sustainable management that emerge from local agreements supported by state and federal government agencies.

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Bioactive peptides in red meats, byproducts and residues from the meat industry

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ABSTRACT

Objective: To provide a comprehensive overview of the generation of bioactive peptides from raw red meat, its by-products, and the waste meat cuts, as well as the bioactive health effects.

Design/methodology/approach: A literature search was conducted through the organization and systematized analysis of information for an updated literature review of bioactive peptides in red meat.

Results: Meat is a food of great nutritional value for human beings, since the digestibility of its protein stands out. In addition to the nutritional value of this protein, there are some studies in meat where bioactive peptides provide bioactivity mainly as an antihypertensive and antioxidant. Bioactive peptides are generally obtained by enzymatic hydrolysis and through microbial fermentation processes. Subsequently, they are identified by analytical techniques to perform *in vitro* and *in vivo* evaluations verifying the bioactivity of the peptides obtained.

Limitations on study/implications: The food industry must establish the most reliable methods for generating more reliable peptides to standardize their production and avoid process variability.

Findings/conclusions: The bioactive activity to be exerted by the peptides will depend on factors specific to the protein, such as the number of amino acids it contains, the hydrophilic or hydrophobic profile, and size, among others. This information is key to predict the activity the peptide can exert in the human body since many can have multiple activities, the most common being the antioxidant-antihypertensive function.

Keywords: bioactive peptides, meat, antioxidant, antihypertensive.

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INTRODUCTION

Meat is a food of great nutritional contribution for human beings, and its absence from the diet can cause diseases related to malnutrition such as metabolism disorders, thrombosis, anemia, among other clinical ailments that affect the population in general [1,

2]. The lack of nutrients that meat consumption contributes, such as high-bioavailability proteins, essential amino acids, and vitamins can affect to a greater extent the sectors of the population that need them most, for example developing children, pregnant and lactating women, as well as the elderly, because they are not easily found in other food groups [3]. Derived from these proteins, smaller peptides are obtained which contribute essential amino acids for the correct function of the body; however, there are peptides with punctual characteristics which, in addition to the nutritional contribution, provide a beneficial activity as antihypertensive, antimicrobial, anti-inflammatory, antidiabetic, hypocholesterolemic, antioxidant, antithrombotic, and anticancer, favoring the consumer's health [4]. The production of bioactive peptides in meat happens naturally during digestion in the gastrointestinal tract, in food processing, or hydrolytically *ex situ* by adding microorganisms or substrates capable of generating proteolytic reactions that reduce the sizes of the protein chains and liberating smaller peptides with bioactive activity [5, 6]. Maturing meat is a necessary process to elaborate meat products, and during this stage the moisture content is reduced, generating breakdown of muscle fibers which affect the length and amount of peptide chains which is reflected in the concentration of bioactive peptides. Currently, the most common ways of conserving meat are storage and transport in refrigeration and/or freezing, and for this reason, it is essential to understand the behavior of the reactions that are generated internally at low temperatures for the generation of bioactive peptides in meat [7, 8].

Therefore, the objective of this study is to gather relevant and updated information about the generation of bioactive peptides in fresh red meats, its byproducts and waste meat cuts such as bones, blood and skin. Through this literature review, a comprehensive description is offered of the bioactive peptides in red meats and of the possible bioactive effects on health.

Peptides in meat and meat products

In particular, red meats are sources of high-quality proteins, contributing on average 20-25 grams of protein for every 100 grams of raw meat, in addition to providing valuable nutrients such as minerals and vitamins, which is why the adequate consumption of meat together with a mixed diet ensures the supply of nutrients [1, 2]. It should be highlighted that the different species destined to the consumption of meat will present different peptide profiles and this is because of factors such as race, sex, age, weight, and metabolic differences between species [9].

Similarly, any conserved and/or processed meat through salting, curing, chemical addition, smoking or any other method that has the benefit of giving added value to the natural cut of meat is considered to be a meat product; and during these processes, the concentration of the water activity is reduced, increasing proteolysis [10]. Meat products are foods that contribute a high content of proteins; however, as a result of the conservation methods and additives commonly used, their salt content is very high and their consumption has been related to health problems like high blood pressure and cancer [11].

The processing of meat products cured dry reduces the activity of water in meat to mature it, and proteolysis begins as a result of the actions of muscular endogenous

peptidases such as calpains and cathepsins, as well as microbial peptidases that generate free amino acids and peptides of low molecular weight [13]. The amount of bioactive peptides produced during the elaboration of a meat product will depend on the physical and chemical treatments which the meat must undergo, such as changes in temperature, time, moisture and pH, since they alter the structures and break the peptide chains, altering the concentration of peptides [14].

Dry-cured meat products as a natural source of antihypertensive and antioxidant peptides are of interest, because they could help to counteract the adverse action of sodium chloride they contain, thus helping to maintain a stable blood pressure and, therefore, a good state of health [2].

Elaborating meat products implies performing some sort of treatment (acid, alkaline, thermic, etc.) on meat, and this process affects the functional and nutritional properties because it modifies the presence of amino acids. Acid treatments destroy asparagine and glutamine, while alkaline treatments destroy cystine, serine and threonine, reducing the presence of bioactive peptides in the final product [15]. The constant variations of temperature and acidity during meat processing also affect the content of bioactive peptides, particularly if sugars have been added since the bioavailability of lysine decreases drastically when Maillard reactions are generated during cooking [9]. Structural changes during the oxidation of meat proteins and meat products can alter the rate of hydrolysis and affect the bioavailability of amino acids, and consequently, the yield of bioactive peptides will be low [12].

Methods for obtaining bioactive peptides

Because peptides are encrypted in a mother protein, the techniques to produce them should be specific and selective to promote an adequate liberation of peptides; it can be done through the action of endogenous enzymes during the processing, the gastrointestinal digestion in the consumer's tract, or the use of commercial exogenous enzymes [16]. There are also peptides that are produced by bacteria, which generate two variants of peptides, ribosomal peptides and non-ribosomal peptides, the latter obtained from enzymes and multi-enzymatic complexes [17]. In the industry, protein hydrolysis can be done with acids, alkali metals or enzymatic agents to generate bioactive peptides from meat, meat products and byproducts [18]. The use of endogenous proteins, that is, those from meat, is limited to certain products such as those cured and matured since the activity of these enzymes is limited and the yield of obtaining peptides is relatively low [19]. Therefore, the use of exogenous enzymes that have a greater activity is preferred, which are extracted from plants, microbes or animal tissues. The selection of dietary proteins from which peptides will be obtained is determined through the criteria of whether they come from a byproduct that can give added value and if the production of such peptides has a pharmacological interest [20].

Different exogenous enzymes have specificity for proteolysis, the selection of the correct enzyme will influence the sequence of amino acids from peptides that give rise to different nutritional, functional and bioactive properties in the hydrolysates [21, 22].

The most frequently used traditional techniques for the generation of peptides in the industry are hydrolysis and fermentation, from which free amino acids and short-chain peptides are obtained, and the ultra-filtration is also used when an estimated molecular weight of the peptide fractions is sought. When it comes to peptide purification, high-resolution liquid chromatography is the most used technique because a short time is needed and compared to other techniques, it can produce peptides at a large scale; this technique is supported by detectors of ultra-violet (UV) rays and fluorescence for the detection of peptides [23, 24].

The peptides are obtained from proteins through *in vivo* and *in vitro* hydrolysis or during the fermentation of foods using proteolytic cultures [20]. In addition, the hydrolysis of meat proteins results in polypeptides with a smaller number of amino acids than the original protein, and when hydrolysis continues, smaller peptides are liberated until they reach tripeptides, dipeptides, and free amino acids [25].

A low-cost technique of bioactive peptide production used is microbial fermentation using feed-grade strains where microorganisms liberate proteases to hydrolyze extracellular proteins that they use as source of nitrogen, resulting in the liberation of short-chain peptides and although their use is focused on dairy products, they also give good results in meat products such as chorizo [26, 27].

Identification of bioactive peptides

To identify the sequences of peptides, it is fundamental to analyze the protein sequences. For this purpose, a variety of techniques are used jointly to achieve better results, the most frequent combination being liquid chromatography and mass spectrometry [28].

In fermented products, the production and characterization of new peptides will depend on the type of substrate, microbial strain, proteolytic enzyme, and conditions that must be guaranteed while obtaining them, such as pH, time-temperature rate, and enzyme/substrate concentration which optimize the maximum activity of the enzyme to obtain a higher yield. However, this methodology lacks standardized protocols for the evaluation of biological activities [21, 29].

Although microbial fermentation and enzymatic hydrolysis are the most demanded techniques for the production of bioactive peptides due to their relatively simple and safe processes, the thermal processes should be supervised to avoid alterations in the structures of amino acids such as racemization, decomposition, dehydration (primarily serine and threonine), cycling of Beta eliminations, or glycation, because they can affect the functionality of peptides [30].

In addition to the techniques to obtain bioactive peptides of natural origin, it is also possible to synthesize them through chemical, enzymatic or recombinant DNA synthesis if their amino acid composition is known [31].

Various methodologies have been used to identify the residues generated by proteolysis of the muscle proteins; the use of mass spectrophotometry in tandem is the most used technique to determine the peptide sequences generated during the natural processes of protein hydrolysis; electrophoresis in polyacrylamide gel helps to separate larger proteins

and to identify them through fingerprints of peptide masses with matrix-assisted desorption/ionization laser mass spectrophotometry [32].

Reversed-phase high-performance liquid chromatography is the technique that is mostly used to characterize the sequences of bioactive peptides and together with mass spectrometry it can undo the sequence by molecular weights; despite being a viable solution, the costs are high so other purification methods have been chosen such as ultrafiltration, gel chromatography, ionic exchange, and exclusion by size, among others [14]. After synthesizing the bioactive peptides, the greatest problem for the industry is the application in foods, because of the bitter taste they have, low stability in food matrices and susceptibility to gastrointestinal digestion.

New techniques to obtain and to process peptides emerge as scientific discoveries advance, which have shown to be environment-friendly, sustainable and efficient; among them, there is extraction assisted by microwaves and by ultrasound. Regrettably, the costs of specialized equipment are too high, so standard techniques which have been given good results for years are still being used [24].

Another of the methodologies implicated for the identification of residues generated in proteolysis is the *in silico* predictive analysis, which allows detecting the bioactive peptides of several proteins with the help of databases to track the original proteins from which short amino acid sequences were originated and which also help to predict the potential biological activity that they can have [14].

To evaluate *in vivo* the stability of bioactive peptides during digestion is a complicated task, since doing it represents experimenting in humans which can be invasive and uncomfortable [6, 33]. For this reason, *in vitro* digestion tests are carried out to evaluate the stability and the actual beneficial activity of the peptides, in simulated digestions tests where scenarios are set out that imitate the conditions of a digestive system, such as the enzymes and the optimal time [34]. This represents one of the greatest challenges for the food science branch, because of the inability for a greater amount of bioactive peptides to remain intact through the digestive tract. Despite this, there are promising peptides such as lactotriptides Ile-Pro-Pro and Val-Pro-Pro which have proven to keep their native structure, and they are being tested in commercial products [35].

Meat wastes

In the meat industry, animal waste byproducts are generated while obtaining meat, such as esquilmos (parts of the animal that are not used to elaborate meat products), and secondary meat cuts abundant in fat with low commercial value, among others [36].

The blood, non-edible organs, skin, horns, hoofs, bones, fat tissues are considered useless elements for the large trade of meat cuts, which does not mean that they are not useful; the functional value of their proteins continues to be very high since there are proteins of animal origin from which protein concentrates can be obtained with multifunctional activities such antioxidant and antihypertensive [37, 38]. In rigid structures such as connective tissue, bones and cartilage from vertebrates there is a functional fibrous protein complex that makes up close to a third of all the body proteins called collagen; its structure is made up mainly by glycine molecules and other amino acids in three intertwined chains

forming a triple helix, which can be split by exogenous proteases to generate bioactive peptides in foods, and the main application is in gelatin elaboration [21]. In a study carried out with collagen obtained from pork skin, it was identified that the sequence Gln-Gly-Ala-Arg has positive effects when the oxidation speed of linoleic acid and the elimination of free radicals slow down [39].

The main technique to obtain hydrolysates from this structure is enzyme proteolysis and studies have been centered in their antioxidant and antihypertensive activity because of its constant sequences of Gly-Pro-Hyp that can unleash these potential biological activities [38]. The aromatic amino acids tyrosine, tryptophan and phenylalanine have the capacity to act as eliminators of radicals. In particular, the antioxidant activity of tyrosine associated with the ability of phenolic groups to act as hydrogen donors, thus deactivating the free radicals [40]. Antioxidant peptides with leucine in their N-terminal carbon, such as Leu-Asp-Gln-Trp, Leu-Pro-His-Ser-Gly-Tyr and Leu-Leu-Gly-Pro-Gly-Le-Thr-Asn-His-Ala, can improve the capacity to capture electrons in the peptide interaction while other dipeptides mostly identified as histidine, carnosine and anserine have been highlighted because of their demonstrated antioxidant activity [2, 14].

Table 1. Antioxidant bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
DSGVT; IEAEGE; DAQEKLE	Porcine muscle <i>longissimus dorsi</i>	Saiga, Tanabe y Nishimura [44]
DLYA; SLYA; VW	Porcine muscle	Arihara y Ohata [45]
ALTA; SLTA; VT	Porcine muscle	Arihara [46]
NR	Porcine blood	Chang <i>et al.</i> [47]
QGAR	Pork meat	Li <i>et al.</i> [39]
NR	Porcine plasma	Wang <i>et al.</i> [48]
MQIFVKTLTG	Deer muscle	Kim <i>et al.</i> [49]
NR	Porcine plasma	Xu <i>et al.</i> [50]
NR	Pork meat	Liu <i>et al.</i> [51]
QYDQGV; YEDCTDCGN; AADNANELFPPN	Buffalo horn	Liu <i>et al.</i> [52]
NR	Pork liver	Di Bernardini <i>et al.</i> [53]
FGG; DM	Pork sausage	Broncano <i>et al.</i> [54]
NR	Porcine blood	Alvarez <i>et al.</i> [55]
SAGNPN	Pork cured ham (Landrace-Large White)	Escudero <i>et al.</i> [56]
QYP	Fermented ground pork sauce	Ohata <i>et al.</i> [57]
DLEE	Xuanwei cured pork ham	Xing <i>et al.</i> [58]
GKFNV; LPGGGHGDG; LPGGGT; HA	Jinhua pork ham	Zhu <i>et al.</i> [59]
SNAAC	Pork cured ham (Landrace-Large White)	Gallego <i>et al.</i> [60]
AEEEYPDL	Pork cured ham (Landrace-Large White)	Gallego, Mora y Toldrá [61]

NR: Unreported peptide sequences.

Table 2. Antihypertensive bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
MNPPK; ITTNP	Porcine Myosin Thermolysin Hydrolysate	Arihara <i>et al.</i> [62]
ITTNP	Hydrolyzed porcine myosin thermolysin hydrolysate	Arihara <i>et al.</i> [62]
RMLGQTPTK	Pork loin (<i>Longissimus dorsi</i>)	Katayama <i>et al.</i> [63]
VLAQYK	Beef meat	Jang <i>et al.</i> [64]
VLAQYK	Beef meat	Jang <i>et al.</i> [65]
SPLPPE; EGPOGPPGPVG; PGLIGARGPPGP	Beef muscle	Bauchart <i>et al.</i> [66]
LGFTTKTYFPHF; VVYPWT	Pork blood	Yu <i>et al.</i> [67]
VKKVLGNP	Pork meat	Katayama <i>et al.</i> [68]
GFHI; DFHING; FHG; GLSDGEWQ	Beef	Jang <i>et al.</i> [69]
KRQKYDI	Pork meat	Katayama <i>et al.</i> [70]
KRVITY	Pork meat	Muguruma <i>et al.</i> [71]
KAPVA	Pork long back muscle	Escudero <i>et al.</i> [72]
TKAVEHLDDLPGALSELSDLHAHKLRVDPV NFKLLSHSL; LDDLPGALSELSDLHAHKLRVDPVNFKLLS HSL; KLLSHSL; LLSHSL	Beef blood	Adje <i>et al.</i> [73]
AKGANGAPGIAGAPGFPGARGPSGPQGPSGPP; PAGNPGADGQPGAKGANGAP	Beef calcaneal tendon collagen	Banerjee y Shanthi, [74]
RPR; KAPVA; PTPVP	Pork meat	Escudero <i>et al.</i> [75]
AAATP	Pork cured ham (Landrace-Large White)	Escudero <i>et al.</i> [76]
FQPS	Goat meat	Mirdhayati <i>et al.</i> [77]
NR	Jinhua pork ham	Zuo <i>et al.</i> [78]

NR: Unreported peptide sequences.

On the other hand, thousands of liters of blood are obtained as byproduct from animal slaughter for consumption, and this liquid can represent an environmental problem if it is not managed with due care [36]. Antimicrobial benefits have been found in different studies, mainly from blood, because it is rich in proteins structured by hemoglobin [36]. In the same way, bioactive peptides have been obtained from waste material, through hydrolysis of proteins from skeletal muscle and connective tissues of some species such as pig and chicken [37].

Beneficial activity of bioactive peptides

With the scientific advances it has been discovered that peptides from the food proteins are physiologically active in a direct way, through their presence in the undisturbed food itself or after their liberation during the consumer's digestion, both *in vivo* or *in vitro* [41]. The bioactive compounds accompany the nutritional structures in foods that

provide nutraceutical factors in favor of the consumer's health; they are found in low concentrations so it is necessary to have a constant intake to potentiate their effects [42]. The main intention of generating bioactive peptides is to seek the benefit to human health, although their safety should be verified, preventing them from being allergenic and/or toxic for their consumption, since in most cases they are produced with enzymes that are not found within the human digestive system; in addition, these peptide sequences do not have a record of safe use [21]. A problem that bioactive peptides face is that they must resist the digestion process, be absorbed in the intestinal epithelium, and distributed by the bloodstream to exert their physiological function in the target organ; for example, peptides with proline residues are more resistant to attacks from gastrointestinal enzymes so they can be absorbed in a nearly intact manner [43, 16]. Antioxidant and antihypertensive activities are of special interest in the study because hypertension and cell oxidation play an important role in the development of cardiovascular diseases, which is why the incorporation of bioactive peptides to the diet can prevent these diseases [9].

Table 3. Opioid bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
YPWT; YPFT	Beef blood	Brantl <i>et al.</i> [79]
LVVYPWTQRF; VVYPWTQRF	Beef blood	Piot <i>et al.</i> [80]
Hemorfina 7 LVV & Hemorfina 7 VV	Beef blood	Zhao <i>et al.</i> [81]
LRFPMQR; LVVYPATQR; LVVYPATQRFFE; VVYPATQR; DVGQTVDDPYA; DLHAYKLRVDPVNFKLLSH; FRLLGNVL; VVYPWTQRF; LEGKVLPGVDA; KHVAGAAAAGAVVGSGLGYM; YPWVAD; GVGVAPGVGVAPGIGLPGGVIA	Lamb brain	Ianzer <i>et al.</i> [82]

Table 4. Antimicrobial bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
FLSFPTTKTYFPFDLSHGSAQVKGHGAK	Beef blood	Fogaça <i>et al.</i> [83]
VLSAADKGNVKAAWGKVGGHAAE	Beef blood	Froidevaux <i>et al.</i> [84]
VTLASHLPSDFTPAVHASLKDFLANVSTVL	Beef blood	Daoud <i>et al.</i> [85]
VTLASHLPSDFTPAVHASLKDFLANVSTVLTSKYR; TSKYR; STVLTSKYR; QADFQKVVAGVANALAHRYH	Beef blood	Nedjar-Arroume <i>et al.</i> [86]
SHLPSDFTPA; VHASLKDFLA	Beef blood	Nedjar-Arroume <i>et al.</i> [87]
GFHI; DFHING; FHG; GLSDGEWQ	Beef meat	Jang <i>et al.</i> [69]
KYR; SKYR	Beef blood	Catiau <i>et al.</i> [88]
VNFKLLSHSLLVTLASHL	Beef blood	Hu <i>et al.</i> [89]
YSKYR	Beef blood	Przybylski <i>et al.</i> [90]

Table 5. Antithrombotic bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
NR	Pork meat	Morimatsu <i>et al.</i> [91]
NR	Pork meat	Shimizu <i>et al.</i> [92]

NR: Unreported peptide sequences.

Table 6. PEP-inhibitor bioactive peptides reported by various authors.

Peptide sequence	Source	Reference
MPPPLPARVDFSLAGALN	Bovine brain	Ohmori <i>et al.</i> [93]

CONCLUSIONS

The bioactive activity of peptides depends on factors of the protein, such as the amount of amino acids that it has, its hydrophilic or hydrophobic profile, and size. This information is key to predict the activity that the peptide can exert in the human body, since many of these can have multiple activities, with the most common being antioxidant antihypertensive. The incorporation of meat cuts and inedible parts of animals such as skin, blood, horns and bones to the production of bioactive peptides has opened the possibility of taking the maximum advantage of the consumption of animals for human benefit, and this way to significantly reduce the environmental impact of the industry. The bioactive peptides function as auxiliary in the improvement of the state of health and can also be useful in the treatment of diseases; however, the pharmaceutical industry is facing the challenge of increasing the research on this issue of proteomic applied to medicine, since critical information is unknown to define bioactive peptides as a solution in disease treatment and recovery. Likewise, the food industry should establish methods to generate more reliable peptides to standardize their production and to avoid the existence of variability in the processes.

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LD₅₀ and GR₅₀ estimation with gamma rays (⁶⁰Co) in *Arachis pintoi* Var. amarillo

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ABSTRACT

Objective: To estimate the median lethal dose (LD₅₀) and mean reductive dose (GR₅₀) due to gamma radiation in *Arachis pintoi* var. Amarillo seeds.

Design/methodology/approach: Ten doses were used (100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy) plus a control (without radiation). The experimental design was completely random with three replications and 50 seeds per repetition. Seed germination was evaluated 29 days after sowing (das) and plant survival, plant height, root length and leaf area at 60 das. The LD₅₀ and GR₅₀ for survival and plants height were estimated by linear regression.

Results: There was a significant reduction of seed germination and plant survival from 300 and 200 Gy doses compared to the control (61.64 and 49.15% each); for the plants height the dose was of 100 Gy (35.22%). There were no differences in the root length and leaf area with 100 and 200 Gy regard to the control. The LD₅₀ was estimated at 212.54 Gy and the GR₅₀ at 162.16 Gy.

Findings/conclusions: The gamma radiation doses to induce genetic variation in *A. pintoi* var. Amarillo seeds were between 162 and 212 Gy.

Keywords: *Arachis pintoi*, LD₅₀, GR₅₀, gamma radiation.

INTRODUCTION

Forage production for cattle feeding in the Mexican tropics is mainly carried out in medium to low fertility soils, during seasonal rainfall and four to six months dry season (Ramos and Peralta, 1988). To maintain good forage quality production throughout the year, various legume species are used, including *Arachis pintoi* with an average protein content of 15.1%, and whose forage production is 6.44 t ha⁻¹ year⁻¹, (Cab *et al.*, 2008; Castillo-Gallegos *et al.*, 2014). An important strategy to contribute to the forage production

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with great persistence in the dry season is the development of genetic variation to obtain new *A. pinto* genotypes. With this purpose, different methods including induced mutagenesis have been used (Suprassana *et al.*, 2015).

Before starting an assisted improvement program by induced mutagenesis, it is necessary to know the median lethal dose or the mean reductive dose, which relates to the effective dose to produce genetic variation (Kodym *et al.*, 2012). The results on the determination of the median lethal doses (LD₅₀) and mean reductive doses (GR₅₀) indicates that it is species, variety, and plant tissue specific. Plant genotypes with less genetic redundancy show higher sensitivity to radiation compared with those of greater genetic redundancy (Ukay, 1981).

A. pinto varieties are generated by selection and hybridization methods. However, other improvement methods such as induced mutagenesis have not been explored. This method has allowed the development of mutant variety lines in cereals, flowering plants and legumes (FAO/IAEA, 2016) in short time, compared to conventional methods. Induced mutagenesis is carried out with chemical and physical agents. Still, gamma radiations (physical mutagenic agent) are often used because of several advantages such as the easiness to handle materials after radiation exposure, their availability, reproducibility, uniformity, and ability to penetrate tissues (Mba and Shu, 2012).

Therefore, the objective of this study was to estimate the LD₅₀ and GR₅₀ of gamma rays (⁶⁰Co) in *A. pinto* seeds var. Amarillo and its effect on seed germination, plant survival, plant height, root length and leaf area.

MATERIALS AND METHODS

The irradiation of *A. pinto* var. Amarillo seeds was carried out at the Moscafrut irradiation plant, SADER, located at Metapa de Domínguez, Chiapas, Mexico. An MDS Nordion Gamma Beam 127 panoramic equipment was used, with a 50 g ⁶⁰Co dry storage source and 0.029 Gy s⁻¹ ratio dose. Seeds were exposed to gamma radiation doses of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy, using 150 seeds per dose with 7.8% humidity.

The seed germination, plant survival, plant height, root length and foliar area evaluation of *A. pinto* var. Amarillo was done in greenhouse conditions at the Rosario Izapa Experimental Field located at Tuxtla Chico, Chiapas (14° 40' 16.1" N, 92° 42' 59.1" W, and 435 m altitude). The day after the seed's irradiation, seeding was carried out using 200 cavities (1 × 1 × 4 cm) germination trays, with peat moss[®] as substrate. The experimental design was completely random with three repetitions; each consisted of 50 cavities where a single *A. pinto* seed was placed.

Total seed germination percentage was evaluated 29 days after sowing (das), while the plant survival percentage, plant height, root length and leaf area were evaluated at 60 das; the last of them recorded with a leaf area integrator LI-COR, LI 3100 (three measurements per plant). The variable's record of seed germination and plant survival were done relative to the number of existing plants by repetition; the plant survival percentage was determined with the equation: (number of live plants per repetition/number of plants at 29 das) X 100.

Plant height, root length, and leaf area were assessed on five plants per repetition, on a total of 15 plants per treatment.

The data were analyzed by ANOVA, and the means comparison to the control was tested using the Dunnett test at 95% confidence interval. For plant height, root length and leaf area, only doses of 100 and 200 Gy were analyzed, given the fact that other treatments had less than five plants by repetition. The LD₅₀ and GR₅₀ for plant survival and plant height were estimated using parameters of simple linear regression models, for both analyzes data normality assumptions were checked in each statistical model. All statistical analyses were performed with the SAS statistical software version 9.0 (SAS Institute, 2002).

RESULTS AND DISCUSSION

Seed germination

Gamma radiation influenced seed germination of *A. pinto* var. Amarillo ($P < 0.01$). Doses of 100 and 200 Gy did not affect seed germination compared to the control treatment ($P > 0.05$). The gradual doses increase, in the transition from 200 to 300 Gy, showed the greatest decrease in germination, close to 50%; the 300 to 1000 Gy range showed germination between 16 and 21.33%. These radiation doses provoked reductions from 56.16 to 64.38%, compared to the control (Table 1). High radiation doses affected seed germination in rice (*Oryza sativa* L.) varieties (Pavan-Kumar *et al.*, 2013), *Vigna unguiculata* L. Walp Nakare and Shindimda varieties (Horn and Shimelis, 2013) and *Arachis hypogaea* L. Narayani variety (Aparna *et al.*, 2013). While doses between 5-30 Gy promoted significant increase rate and seed germination percentage in TSh variety corn seeds (Marcu *et al.*, 2014). However, there are also reports that gamma radiation does not significantly affect total germination of soybean Huasteca 100 variety in doses from 100 up to 1250 Gy (Gálvez-Marroquín *et al.*, 2017). Olasupo *et al.* (2016) also argue that the different sensitivity responses to gamma radiation in *Vigna unguiculata* accessions are due to genetic background.

Lokesha *et al.* (1992) reported that the inhibition of seed germination due to radiation effects is attributed to numerous histological and cytological changes, disruption and the tunic's disorganization and cellular division cease at meristematic tissues during germination.

Plant survival

The *A. pinto* var. Amarillo plants survival was negatively affected by gamma radiation ($P < 0.01$). At the 100 Gy dose plant survival was similar to that of the control treatment (92.78 and 98.33%), while at doses from 200 to 400 Gy there was a survival reduction of up to 50%; 100% plants mortality was observed at doses higher than 400 Gy (Table 1). High gamma radiation doses have affected *Laelia autumnalis* protocorms survival (Hernández-Muñoz *et al.*, 2017) and rice (*Oryza sativa* L.) var ADT (R) 47 (Rajarajan *et al.*, 2016). The plant's mortality can be attributed to abnormalities in chromosomes with an increment in the radiation dose. Murugan *et al.* (2015) report an increase, dependent

of radiation dose, in mitotic aberrations of *Catharanthus roseus*, they also observed a dose-dependent reduction in seed germination, plant survival, and plant height. Another factor that contributes to the survival decrease is the gamma photons interaction, particularly with water, which produces free radicals and in turn significant damage to vegetable cells (Kovacs and Keresztes, 2002).

Furthermore, in non-lethal cases, cells have a repair DNA mechanism that can fix different types of damage to a certain accuracy degree (Dexheimer, 2013). Based on this, it will give rise to mutations present more frequently in the second generation, since these are from recessive characters (Foster and Shu, 2012). The main mutations that are present in the second generation are simple base substitutions and insertions/deficiencies, as reported on six M2 rice plants (*Oryza sativa* L.) var. Nipponbare (Li *et al.*, 2016).

Plant height

Gamma radiation significantly affected plant height of *A. pinto* var. Amarillo ($P < 0.05$). The control treatment showed the highest average plant height with 5.28 cm, while the 100 and 200 Gy doses show average values of 3.42 and 2.15 cm each; these correspond to reductions of 35.22 and 59.34% (Table 1). These results concur with those obtained by Taheri *et al.* (2016); they determined a decrease in plant height of *Curcuma alismatifolia* var. Chiang Mai Red, Doi Tung 554, Sweet Pink and Kimono Pink using gamma radiation doses from 14.6 to 87.4 Gy. Hanafiah *et al.* (2016) found a bidirectional response in plant height at the M1 generation of *Hibiscus sabdariffa* L. var. Roselindo 2 with the increase of gamma radiation doses (from 150 to 600 Gy). They did not observe significant effects per dose. However, the maximum value was at doses of 450 Gy. The reduction in plant height has been related to auxins destruction by radiation (Momiya *et al.*, 1999). Although, Ali *et al.* (2016) indicate that gamma rays decrease the growth rate through mutations in the DNA that synthesizes DNA

Table 1. Percentage of seed germination and plant survival, plant height, root length and leaf area of *Arachis pinto* var. Amarillo in function of ^{60}Co gamma radiation dose.

Dose	Germination (%)	Plant survival (%)	Plant height (cm)	Root length (cm)	Leaf area (cm ²)
Control	48.67	98.33	5.28	10.16	28.75
100	44.00	92.79	3.42**	9.51	33.48
200	36.64	50.00**	2.15**	8.79	21.00
300	18.67**	4.44**	-	-	-
400	18.00**	2.56**	-	-	-
500	21.33**	0.00**	-	-	-
600	19.33**	0.00**	-	-	-
700	16.00**	0.00**	-	-	-
800	18.67**	0.00**	-	-	-
900	17.33**	0.00**	-	-	-
1000	17.33**	0.00**	-	-	-

** Significant difference respect to control with 95 % confidence interval.

at the interface, which provoke the interruption of the plant bud and the consequent interruption of cell differentiation.

Root length

Gamma radiation caused no effects on *A. pinto* var. Amarillo root length ($P > 0.05$). Plants in control treatment showed an average root length of 10.16 cm, while in the 100 and 200 Gy doses it was 9.58 and 8.78 cm, each (Table 1). However, in 100 and 200 Gy doses it showed a reduction of 5.7 and 13.5% compared to the control. Verma *et al.* (2017) found a reduction of root length in *Foeniculum vulgare* Mill. in doses of 150 Gy up to 250 Gy, with the lowest value at the maximum dose reduction of 77.21% compared to control. This relates with a reduction in mitotic activity at the roots tips, as reported for *Vigna unguiculata* L. varieties Kaha 1, Azmerly, Cream 7 and Giza 6 at 200 and 300 Gy (Badr *et al.*, 2014).

Leaf area

Gamma radiation did not affect *A. pinto* var. Amarillo leaf area ($P > 0.05$); however, 100 Gy dose increased 16.4% leaf area related to control; while with 200 Gy, the lowest leaf area average value was obtained (20.99 cm²), which was 26.9% less than the control (Table 1). Ramesh *et al.* (2013) reported that the mulberry genotype (*Morus*) Kosen had a 9% leaf area increase with a dose of 100 Gy, compared to control.

LD₅₀ and GR₅₀ estimation

The parameters to determine an optimal dose to induce genetic variation in a genotype of interest are the median lethal and mean reductive doses. In *Arachis hypogaea*, the most economically important species from the *Arachis* genus the median lethal and mean reductive doses to induce genetic variation with gamma radiation are established, as well the particular mix of gamma radiation with EMS for each genotype. In reports from *A. hypogaea* L. var. VRI-2 the estimated LD₅₀ was of 500 Gy + 0.5% of EMS (Gunasekaran and Pavadai, 2015); and the GR₅₀ for local peanuts (*Arachis hypogaea*) type Virginia with 12% humidity required doses of 700 Gy (Brito-Damián and Ángeles-Espino, 2016). In the present study the LD₅₀ for *A. pinto* var. Amarillo plants survival was 212.54 Gy, while the GR₅₀ for height plants was 162.16 Gy (Table 2).

Therefore, gamma radiation doses from 162.16 to 212.54 Gy can be useful to induce genetic variation in *Arachis pinto* var. Amarillo.

Table 2. LD₅₀ and GR₅₀ for plant survival and plant height of *Arachis pinto* var. Amarillo of ⁶⁰Co gamma radiation, estimated by linear regression.

Variable	Pr > F	R ²	Equation	LD ₅₀	GR ₅₀
Plant survival	<0.0001	0.87	Y=99.06130-0.23083x	212.54	-
Plant height	0.0068	0.67	Y=5.18222-0.01567x	-	162.16

CONCLUSIONS

The increase in ^{60}Co gamma radiation dose provokes a tendency in germination decrease, plant survival, plant height, root length and leaf area of *Arachis pinto* var. Amarillo plants. Radiation doses of 100 and 200 Gy have no significant influence in these variables, except for plant survival and plant height. ^{60}Co gamma radiation doses from 162.16 to 212.54 Gy can be useful to induce genetic variation in *Arachis pinto* var. Amarillo.

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Evaluation of the *Genipa americana* L. / *Heliconia stricta* Huber agroforestry system and its effects on soil fertility

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ABSTRACT

Objective: To evaluate the plant development of *Genipa americana* L. and *Heliconia stricta* Huber associated as an agroforestry system, and their effects on soil fertility.

Design/methodology/approach: A completely randomized experimental design with three replications per treatment was used. *Genipa* plantations were established in July 2019 and *Heliconia* in September 2020. Soil samples and analyses were carried out based on the methodologies specified in NOM-021-RECNAT-2000 (SEMARNAT, 2002). Monthly measurements of height, stem diameter, number of leaves, photosynthetic rate were made for *Heliconia* and *Genipa*, as well as number of branches and canopy cover for the latter. Means were compared by Tukey's test at a 5% confidence level.

Results: Contents of organic material (OM), Phosphorus (P) and Manganese (Mn) in the soil did present significant differences because of the treatments, cultivated alone and/or associated, while the contents of Nitrogen (N), Potassium (K), Calcium (Ca), Magnesium (Mg), Zinc (Zn) and Copper (Cu) did not. The behavior and development of *Genipa* was the same in the treatments, while *Heliconia* did present variations.

Limitations on study/implications: It is recommended to continue the study.

Findings/conclusions: The treatments cultivated alone and/or associated improved the (OM) contents in the soil. *Genipa* is not affected by the effect of the treatments evaluated, while heliconias are.

Keywords: *Genipa*, *Heliconia*, Association, Soil, Organic Matter (OM).

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INTRODUCTION

In the tropics, it is common to see combinations of timber-yielding trees as shade for perennial crops (coffee and cacao), disperse trees in agricultural fields and paddocks, as well as trees in lines and under the taungya system (Detlefsen & Somarriba, 2012). These

systems can function as CO₂ sinks (Litynski *et al.*, 2006), for soil and water conservation, and for microclimate modification, among other natural services (CONAFOR, 2012). The tendency towards consumption of what is natural is increasingly stronger since there is a trend in developed countries to change the use of artificial for natural colorant, such as cochineal carmine (Lizárraga & Álvarez, 2019). The International Trade Center (2015) reports that imports in the European Union (EU) of natural dye of plant origin went from 21,842 t in 2006 to 45,876 t in 2015; that is, an increase of 110% in imports. In this sense, *Genipa americana* L. is a tree of economic and ecological importance, its fruit is edible fresh or used for the production of candy, jam, jelly and liquor; various medicinal uses are attributed to it, and the wood can be used for the elaboration of diverse articles and products (Strong and Fragoso, 2006; PIN, 2009; Quesada *et al.*, 2010). However, its greatest potential lies in that its immature fruit contains high levels of iridoids, such as genipin, which is essential for the formation of the blue color as a result of its reaction with primary sources of amines (Bentes & Mercadante, 2014; Fujikawa *et al.*, 1987). It is reported as a species of agroforestry use that contributes benefits to the soil. This is why in this study a new association is suggested as agroforestry system, with *Genipa americana* L. as tree combined with *Heliconia stricta* Huber, which has ornamental potential.

MATERIALS AND METHODS

The study was conducted in the open field of Colegio de Posgraduados, Campus Campeche (19° 50' 13.89" N, 90° 58' 61.11" W) and altitude of 20 masl, locality of Sihochac, municipality of Champotón, Campeche, Mexico. The climate is sub-humid warm with summer rains. The mean annual temperature of the place is 26 °C and the mean annual precipitation is 1,274.7 mm (INEGI, 2009). Planting the species was carried out in plots of 100 m².

Establishment of the plantation

Prior to planting, minimum tillage was conducted on the piece of land and three treatments with two repetitions were established in each plot of 100 m²: associated plantation of *Genipa americana* L. with *Heliconia stricta* Huber (T1), monocrop of *Genipa americana* L. (T2), and monocrop of *Heliconia stricta* Huber (T3).

Planting *Genipa americana* L. (Ga) was carried out in July, 2019, at a distance of 3×2 m between furrows and plants, respectively (Figure 1). Planting *Heliconia stricta* Huber (Hs) was conducted in October 2019 at a distance of 2×1 between furrows and plants, respectively (Figure 1), and interspersed at 0.50 m of distance from the Ga lines (Figure 1). However, they did not survive because of lack of irrigation in the spring of that year. Therefore, the crop of this species was established again in September, 2020. The experiment was established in a completely randomized design (CRD) with three repetitions per treatment, planting date, to analyze the variables of height, stem diameter and number of leaves, photosynthetic rate in Hs and Ga, plus number of branches and green plant cover in Ga. The data were analyzed as a CRD with subsampling (Zamudio and Alvarado, 1994), using the statistical software InfoStat. The multiple means comparison test was carried out with Tukey's method with a significance level of 5%.

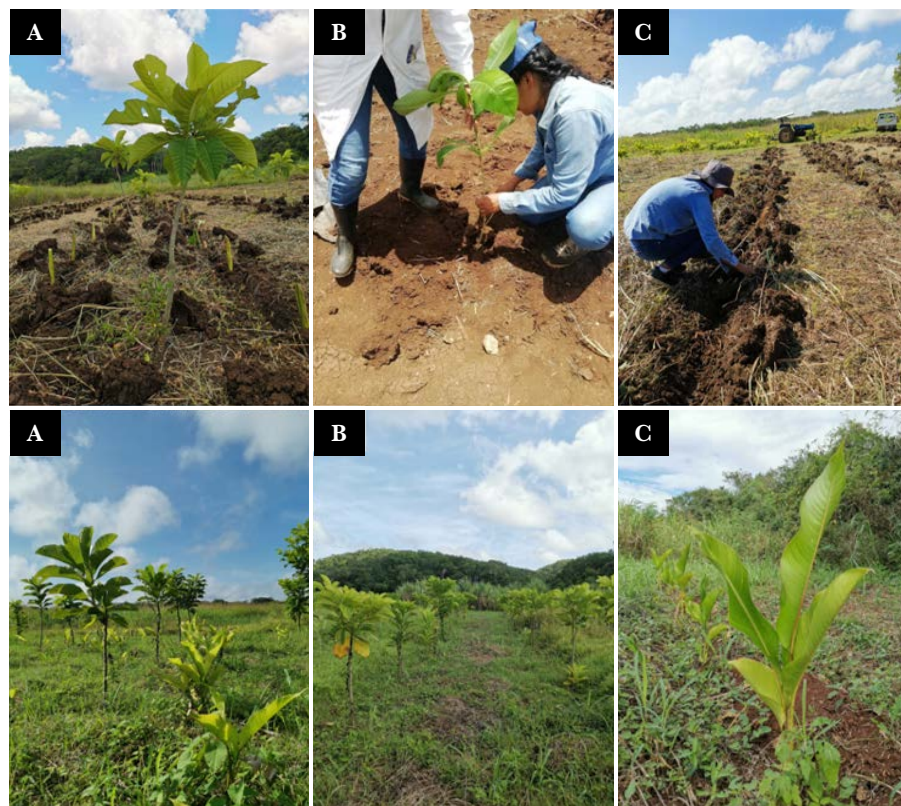


Figure 1. Establishment of the treatments: (T1) Plantation associated of *Genipa americana* L. with *Heliconia stricta* Huber, (T2) monocrop of *Genipa americana* L., and (T3) monocrop of *Heliconia stricta* Huber.

Soil sampling and analysis

The samples and analysis of the soil were obtained as described in NOM-021-REC/NAT-2000 (SEMARNAT, 2002). A first sample of the entire plot was taken in July, 2019. The second sampling was carried out per treatment and the repetitions in March of 2021. The following were determined: pH, OM, N, P, K, Ca, Mg, Zn and Cu.

Sampling of the species in the treatments

Sampling in *Genipa americana* L.

Monthly measurements of the variables were carried out as follows: height of the plant's stem or shaft with the help of a measuring tape; stem diameter with a Vernier at the height of the base of the stem; number of leaves per plant, by counting considering all the true leaves on the plant; number of branches per plant considering all the branches on the plant; photosynthetic rate, which was determined using the LI-6400X equipment to measure photosynthesis (Carmona *et al.*, 2007); and canopy cover which was measured with the mobile phone application Canopeo (Jáuregui *et al.*, 2019).

Sampling in *Heliconia stricta* Huber

To understand the behavior of the development of *Heliconia stricta* Huber, the following variables were evaluated monthly: plant height, measured with a measuring tape, from

the base of the plant to the tip; number of leaves per plant, counting the number of leaves considering all the true leaves on the plant; and photosynthetic rate.

RESULTS AND DISCUSSION

The soil, as a result of the effect of the *Heliconia stricta* Huber and *Genipa americana* L. crops

According to Table 1, the contents of N, K, Ca, Mg, Zn and Cu in the cultivated soil did not have significant differences as a result of the effect of treatments in single and associated crops; the contents of OM, P and Mn did present differences from the effect of such treatments, as well as the pH.

When it comes to the nutritional diagnosis of the soil in the treatments (T_i, T1, T2 and T3) according to what was established in SEMARNAT (2002), a high content was found in bases exchanged (K, Ca, Mg), which coincides with studies carried out by Fernández-Ojeda *et al.* (2016) in the systems they evaluated. In terms of the content of micronutrients (Mn, Zn, Cu), normal levels were found, in contrast with what was reported by Méndez (2013), who found medium levels when he evaluated similar treatments in the field.

The organic matter had an increase, going from a medium to a high percentage, which can be attributed to the contributions of fallen leaves, root fragments, and other plant components incorporated into the soil of the treatments (T1, T2 and T3) in the growth process. Studies carried out in the soil with agroforestry management show that in the superficial layer of the soil there are larger amounts of OM (Arévalo-Gardini *et al.*, 2015), due to residues from pruning, weeding and other factors (Hameed *et al.*, 2018). Regarding the content of phosphorus, it went from medium to low levels, results that agree with what was found in the systems evaluated by Fernández-Ojeda *et al.* (2016), who reported a low

Table 1. Chemical diagnosis associated to soil fertility before and after the treatments.

Determination	Unit	Treatment			
		T _i	T1	T2	T3
pH		6.97 a	7.83 a	7.64 a	7.51 a
MO	%	3.32 b	4.80 a	4.12 a	4.17 a
N		0.17 a	0.19 a	0.17 a	0.17 a
P	(mg kg ⁻¹)	6.95 a	2.01 b	1.44 b	2.68 b
K	(Cmol (+) kg ⁻¹)	0.63 a	0.77 a	0.99 a	0.88 a
Ca		26.77 a	33.85 a	33.34 a	29.06 a
Mg		4.25 a	3.79 a	4.49 a	4.59 a
Mn	(mg kg ⁻¹)	285.30 a	35.43 b	54.36 b	40.85 b
Zn		1.00 a	0.38 a	1.24 a	0.44 a
Cu		2.11 a	1.94 a	2.15 a	2.14 a

Means followed by the same letter in each line do not show significant differences as a result of the effect of treatments (Tukey, $\alpha=0.05$). T_i=Initial condition of the soil before establishing the treatments (capacity of nutritional supply for the crops exhibited by the soil). T1=Condition of the soil containing the treatment of the association *Genipa americana* L./*Heliconia stricta* Huber. T2=Condition of the soil containing the treatment of *Genipa americana* L. T3=Condition of the soil containing the treatment of *Heliconia stricta* Huber.

content of this nutrient as a result of the effect of the cultivation systems evaluated, due to the demand of this element by those cultivars, since it requires being mineralized to be used by plants, under the influence of temperature, pH, aeration, the nature of organic matter, and the rate of carbon/phosphorus (Alcántar-González *et al.*, 2016). When it comes to the content of Manganese, a decrease was found as a result of the effect of treatments T1, T2 and T3 compared to T_i; however, the content of that nutrient in the cultivated soil continues to be adequate (SEMARNAT, 2002).

The soil pH showed changes in values considered as neutral in the conditions of T_i to moderately alkaline in the cultivation systems established (T1, T2 and T3), showing the influence of the pH on the availability of soil nutrients for the plant and the functions that it has in the chemical and biological activity of the soil (Larson and Pierce, 1991; Doran and Parkin, 1994). It is important to highlight the importance of this chemical property on the availability of soil nutrients for the plants cultivated, which is why Alcántar-González *et al.* (2016) mention that in neutral or alkaline pH, the decomposition of the OM from bacteria and actinomycetes predominate; that alkalinity promotes the deficiency of Mn, since it favors its chemical and bacterial oxidation, reducing its exploitation as could happen in certain stages in flooded soils (Silva, 2007); it also fosters the mineralization of organic P in the soil to a greater extent (Tabatabai and Dick, 1979). This behavior of the soil pH as a result of the effect of treatments applied in the field is of great relevance in agricultural systems of the Yucatan Peninsula, if it is considered that soils of this peninsular region are calcareous in much of the eco and agro ecosystems, because of their geological nature.

Growth and development of *Genipa americana* L.

No significant differences were found in the variables evaluated in *Genipa americana* L. from the effect of the treatments evaluated (Table 2), since in both treatments the plants develop in the same proportions, so it can be said that they are apt for their plant association in agroforestry management, as mentioned by García and Islas (2018).

Growth and development of *Heliconia stricta* Huber

As Table 3 shows, the variable plant height of *Heliconia stricta* Huber presented significant statistical differences, where the greatest height was in T1=16.84 compared to T3=15.63 as a result of the effect of treatments. Therefore, it is inferred that the sum of diverse factors, among them the shade favored by *Genipa americana* L. to the *Heliconia stricta* Huber plants, have an effect on the growth of the latter, as well as on the availability of nutrients present in the soil, among other effects.

Table 2. Statistical averages of the variables evaluated in *Genipa americana* L.

Treatment	Height (cm)	Diameter (mm)	Number of branches per plant	Number of leaves per plant	Photosynthesis (mmol CO ₂ m ⁻² S ⁻¹)	CANOPEO
T1	96.40 a	20.45 a	1.75 a	22.00 a	15.64 a	59.52 a
T2	95.88 a	20.80 a	1.71 a	22.58 a	13.40 a	59.97 a

* Means followed by the same letter by column (plant age) do not show significant differences from the effect of treatments (Tukey=0.05). T1=Agroforestry association *Genipa americana* L. / *Heliconia stricta* Huber. T2=Monocrop of *Genipa americana* L.

Table 3. Statistical averages of the variables evaluated in *Heliconia stricta* Huber.

Treatment	Height (cm)	Diameter (mm)	Number of leaves per plant	Photosynthesis (mmol CO ₂ *m ⁻² *S ⁻¹)
T1	16.84 a	11.19 a	2.95 b	7.67 b
T3	15.63 b	10.81 a	3.52 a	9.23 a

* Means followed by the same letter by column do not show significant differences as a result of the effect of treatments (Tukey=0.05). T1=Agroforestry association *Genipa americana* L. / *Heliconia stricta* Huber. T3=Monocrop of *Heliconia stricta* Huber.

According to Uc-Ku *et al.* (2019) and Baltazar-Bernal *et al.* (2020), mycorrhizal colonization in *Heliconia stricta* improves plant height, which is very similar to what was found in Pinto (2007) where two species of heliconias presented a greater height when organic fertilization was applied, in contrast with the application of mineral fertilizer, showing the same development than the control treatment. On the other hand, the stem diameter for *Heliconia stricta* Huber did not present statistically significant differences as a result of the effect of treatments, since they developed equally in both treatments. When it comes to the number of leaves, there were statistically significant differences in treatments T1 and T3, with a larger number of leaves in T3=3.52 compared to T1=2.95, which could be due to competition over nutrients in T1.

Authors like Neto *et al.* (2011) point out that under open field conditions and the application of chemical fertilization, *Heliconia stricta* is favored in plant development, although at the same time it reduces the production of inflorescence, and that without fertilization there is a better inflorescence; therefore, we can relate it with the good content of OM in the soil. Concerning the photosynthetic rate in the months evaluated, statistically significant differences were found as a result of the effect of the treatment, where the highest photosynthetic rate was found in T3=9.23 compared to T1=7.67 (Table 4). On the other hand, Uc-Ku *et al.* (2019), in their evaluation of photosynthesis in *Heliconia stricta* Huber in different samples of the effect of mycorrhizal inoculation, obtained lower values of photosynthesis in the species without inoculation under greenhouse conditions, and therefore, it can be noted that the species increases its photosynthetic activity at plain sunlight.

CONCLUSIONS

Both the association and the establishment of each plant species as monocrop did not affect the contents of N, K, Ca, Mg, Mn, Zn and Cu in the soil. The contents of OM improved, and the P suffered a decrease from the demand of the plants cultivated. It is feasible to associate *Genipa americana* L. in agroforestry management. More studies are required in *Heliconia stricta* Huber on its management in agroforestry systems.

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Alimentary and anti-methanogenic potential of four species of tropical fodder legumes in domestic ruminants

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ABSTRACT

Objective: To describe some considerations about the alimentary and anti-methanogenic potential of *Bauhinia divaricata*, *Dalbergia glabra*, *Piscidia piscipula* and *Caesalpinia vesicaria* (Fabaceae) present in Campeche, Mexico, in domestic ruminants.

Design/methodology/approach: The information was obtained from databases and archives in the internet, as well as official websites of national and international organizations.

Results: Although there is vast information about some Fabaceae species, it is still limited for *B. divaricata*, *D. glabra*, *P. piscipula* and *C. vesicaria*, although they are consumed by domestic ruminants. The few studies available report between 11 and 18% of raw protein (RP) and some secondary metabolites; however, there are no studies that allow understanding their anti-methanogenic potential and their effects on productivity.

Limitations on study/implications: This study offers a panorama of the alimentary and anti-methanogenic potential of four tropical species in domestic ruminants.

Findings/conclusions: *B. divaricata*, *D. glabra*, *P. piscipula* and *C. vesicaria* grow in the Yucatan Peninsula, they are found in grazing zones and are consumed by animals; however, there are few reports that determine the nutritional value of their edible components and no reports that evaluate their effect on productivity of domestic ruminants or their capacity to decrease the ruminal production of methane (CH₄).

Keywords: cattle; fodder; secondary metabolites; tropical trees; methane.

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INTRODUCTION

Livestock production is facing diverse environmental challenges, among which greenhouse gas emissions (GGE) stand out, with the region of Latin America and the Caribbean being one of the zones of highest GGE production in the world, primarily from beef production (FAO, 1997). This is because an important number of domestic ruminants in this region are fed with low-quality fodders, especially during the drought period. During this period, grasses decrease their content of raw protein (RP) and increase the content of neutral detergent fiber (NDF), limiting the ruminal fermentation of dry matter (DM) and the absorption of volatile fatty acids (VFAs), in addition to increasing the retention time

of the feed. As consequence, the production levels of the animals are low but the amount of ruminal methane (CH₄) produced is high (Ku-Vera *et al.*, 2020). This is worsened in tropical regions where grasses of photosynthetic path C4 predominate, since the livestock fed with these grasses produces more CH₄ by kg of DM consumed than the livestock fed with C3 grasses, typical of temperate or cold regions (Thompson and Rowntree, 2020). Therefore, it is essential to decrease the environmental impact of livestock activities in tropical regions, with the diet being a way to achieve it, primarily with the use of local fodder trees.

There is information about the nutritional composition of diverse fodder trees such as *Leucaena leucocephala*, *Guazuma ulmifolia* or *Gliricidia sepium*, as well as their effects on ruminal fermentation and CH₄ production, both in *in vitro* and *in vivo* studies (Piñeiro-Vázquez *et al.*, 2015; Naranjo *et al.*, 2016; Molina-Botero *et al.*, 2019; Galindo *et al.*, 2016; Castrejón-Pineda *et al.*, 2016; Canul-Solis *et al.*, 2020). However, there are several tropical species (Fabaceae) that have been scarcely studied despite being present in the grazing areas and consumed by the livestock. Because of this, the objective was to describe some considerations about the alimentary and anti-methanogenic potential of *Bauhinia divaricata*, *Dalbergia glabra*, *Piscidia piscipula* and *Caesalpinia vesicaria* present in Campeche, Mexico, which are consumed by domestic ruminants.

MATERIALS AND METHODS

The search for information began in August 2020 and ended in March 2021, through databases and data archives available on the internet, among which Google Scholar, Redalyc, SciELO and NCBI stand out, as well as official websites of national and international organizations such as CICY and FAO. Terms in Spanish and English were included, individually or combined, including words like: “ruminants”, “domestic ruminants”, “bovines”, “ruminal methanogenesis”, “methane”, “greenhouse gases”, “legumes”, “fodder legumes”, “tropical legumes”, “*Bauhinia divaricata*”, “*Dalbergia glabra*”, “*Piscidia piscipula*” and “*Caesalpinia vesicaria*”. The information was analyzed and synthesized to extract the key points that allowed fulfilling the objective of this literature review.

Fodder legumes (Fabaceae)

The Fabaceae family is one of the three vascular plant families best represented globally; 737 genera and 19,325 species of cosmopolitan distribution are recognized, primarily in warm and temperate regions. In Mexico, there are 139 genera and 1850 species of this family (Rzedowski *et al.*, 2016). The fodder species of legumes (Fabaceae) in association with grasses (Poaceae) play a relevant role in the nutrition and sustainability of livestock systems, due to their higher content of RP (up to 25% in dry base) and lower content of cell walls than the commonly used grasses in domestic ruminant diets. In addition, they have a higher passage rate because they are highly fermentable and have less retention time in the rumen, decreasing the methanogenesis (Ku-Vera *et al.*, 2020); however, there are other species that have been scarcely used, due to factors such as scarcity of commercial seed or lack of knowledge of their management, so the information regarding their nutritional

potential and their effect on ruminal fermentation is limited (Sosa-Montes *et al.*, 2020). The characteristics of four fodder species found on the roads or paddocks where livestock graze in Campeche, Mexico, are described.

***Bauhinia divaricata* L.** It is a shrub or tree up to 8 m tall (Figure 1a). It is commonly known as tatil bichim (Huasteco), pata de res, pata de vaca, pata de venado, pie de cabra (Rzedowski and Calderón de Rzedowsky, 1997); in Maya as chandzulutok, cocohof, dsuruktok, ts'ulubtok, turku-tov, xdzuruntok, xpata, vaca-xmaywakax (Torres-Colín *et al.*, 2009). In general, it is found in low deciduous forest, medium sub-deciduous forest, and sub-evergreen forest. It also penetrates derived secondary vegetation. It is distributed in warm and humid to sub-humid lands of nearly all of Mexico, extending to Costa Rica and the Antilles (Rzedowski and Calderón de Rzedowsky, 1997). Among the uses of *B. divaricata*, the use of wood is reported for construction of houses, palapas, chicken coops and other rural constructions; in traditional medicine, it is used against stomach pain, bronchitis, asthma, colitis and snake bites (Torres-Colín *et al.*, 2009). Heike (2010) describes it as a perennial ruderal plant since it grows on the edge of roads and disturbed environments. It can also be found in paddocks and tropical plantations used as fodder; occasionally, it is used as ornamental and considered an important honey-producing species. In Yucatán the interior bark is used to make traps and fences.

There is scarce information regarding its use in livestock feed. In a study carried out by Sosa-Rubio *et al.* (2004), where the fodder potential of tropical trees and shrubs in the state of Quintana Roo was evaluated, the authors reported different uses of *Bauhinia divaricata*, among them as fodder, shade and ornamental. In that study, they report that there is 13.7% of RP, 48.0% of NDF and 34.7% of acid detergent fiber (ADF), as well as 38% of *in vitro* digestibility of DM (IVDDM).

Cab-Jiménez *et al.* (2015), in a study carried out in Campeche about the chemical composition and digestibility of fodder tropical trees, found values of IVDDM at 72 hours of 67.1%, as well as 16.9% content of RP, 60.9% of NDF and 35.4% of ADF during the drought season. On the other hand, there is scarce information about the content of secondary metabolites. In this regard, Albores-Moreno *et al.* (2018) reported 3.8% of condensed tannins, as well as presence of alkaloids and saponins. The RP content and of secondary metabolites, as well as its high IVDDM, make *Bauhinia divaricata* an excellent alternative for the diet of domestic ruminants; however, there are few studies and there are no reports of their use in *in vivo* studies. There are also no reports about its potential to decrease ruminal methanogenesis.

***Dalbergia glabra* (Mill.) Standl.** It is a tree or shrub (Figure 1b) that is distributed in the states of Chiapas, Oaxaca, Tabasco and Veracruz, primarily in high evergreen forest, low deciduous forest, medium sub-evergreen forest, and in secondary vegetation, with interest as a honey-producing and timber-yielding species (CICY, 2010). In Campeche, this species is commonly seen in paddocks and natural vegetation, as well as disturbed areas. The information available regarding its use in livestock feed is practically inexistent. A study carried out by López-Herrera *et al.* (2008) about native fodder plants in Ejido Kantunilkin, in Quintana Roo, Mexico, reported that *D. glabra* is known by producers, hunters and practitioners of traditional medicine with the

common name “verde” and it is a species with fodder potential in this zone. In that same study, the authors reported a content of RP, NDF and ADF of 18.7%, 62.9% and 41.5%, respectively, as well as presence of saponins, phenols and alkaloids. The high content of RP and the presence of various secondary metabolites suggest that *D. glabra* could have the potential to decrease ruminal production of CH₄; however, there are no studies about it. There are also no studies that assess its effects on ruminal fermentation or on livestock productivity.

***Piscidia piscipula* (L.) Sarrg.** It is known with the common name of jabín, habín or ja’abin (Maya) (CICY, 2010). It is a tree of 15 to 26 m of height and 57 to 62 cm of diameter (Figure 1c) (Vester and Navarro, 2007). It is one of the species with highest density and relative dominance of the plant community in Campeche, Mexico (Zamora-Crescencio *et al.*, 2014). In the Yucatan Peninsula, it is considered a nectar-polliniferous, fodder, medicinal and ornamental plant. In addition, its leaves and wood are used in the elaboration process of Pib, required to prepare Pibil, a specialty of traditional Mayan cuisine (Zamora-Crescencio *et al.*, 2009). Regarding its potential as feed for livestock, there are some studies that show the chemical and nutritional composition of *P. piscipula*. In this regard, Cab-Jiménez *et al.* (2015) reported that it has 18.3% of RP, 52.9% of NDF and 40.7% of ADF, close to 60% of IVDDM and did not show presence of saponins in samples collected during the dry season. Authors like Albores-Moreno *et al.* (2018), in a study carried out in fodder trees of secondary vegetation (Acahual), reported values



Figure 1. Four species of tropical fodder legumes from Campeche: a. *Bauhinia divaricata*; b. *Dalbergia glabra*; c. *Piscidia piscipula*; d. *Caesalpinia vesicaria*.

of 15.5%, 55.3% and 44.8% of RP, NDF and ADF, respectively, as well as 44.2% of IVDDM, 2.6% condensed tannins and abundant presence of saponins and alkaloids. Meanwhile, the study by Sosa-Rubio *et al.* (2004) reported a content of 11.5%, 61% and 47.8% of RP, NDF and ADF, respectively, as well as 51% of IVDDM. Likewise, López-Herrera *et al.* (2008), reported 12.6% of RP, 50% of NDF and 34.6% of ADF, as well as presence of saponins and phenols. The previous results indicate that *P. piscipula* could be a species used in paddocks, not only as live fences or shade for the livestock, but also as feed due to their contribution of RP and adequate digestibility. Likewise, due to its content of secondary metabolites, it could modify ruminal fermentation and decrease the production of CH₄, although it would be necessary to conduct studies about it.

***Caesalpinia vesicaria* L.** It is a species (Figure 1d) known as mareña (Spanish) or ya'ax k'iin che' (Maya) (CICY, 2010). It is a species of the dry forest (0-400 masl), which is distributed from Mexico to Nicaragua. It has various uses such as firewood, carbon, rustic constructions, beams, bridges, fence posts, and ornamental. Pulverized carbon from the bark is a domestic remedy against diarrhea in children (Quezada *et al.*, 2010). It is also used for the construction of rustic houses, in live fences, and for posts because its wood is very hard and resistant. Until now, there are no studies that indicate its nutritional contribution or value; however, it is reported as a species rich in tannins (CICY, 2010). Of the four species reported in this review, *C. vesicaria* is the least studied, so there is an area of opportunity to evaluate its viability in the diet of ruminants in Campeche.

CONCLUSIONS

There is enough evidence that shows that several species of the Fabaceae family have a high nutritional value and the capacity to decrease the production of CH₄ in ruminants because of the contents of secondary metabolites present in the edible parts of the plant. It is possible for other species of this botanical family to have the same alimentary and anti-methanogenic capacity. Presently, the need to apply strategies to decrease CH₄ emissions from ruminal fermentation is urgent, and these fodder species can be an option for domestic ruminants under grazing in tropical regions. The species *Bauhinia divaricata*, *Dalbergia glabra*, *Piscidia piscipula* and *Caesalpinia vesicaria* grow in the Yucatan Peninsula, they are found in grazing zones, and are consumed by livestock; however, the reports that determine the nutritional value of their edible components are scarce and there are no studies that evaluate their effect on the productivity of the animals and on their capacity to decrease the ruminal production of CH₄. Therefore, there is an area of opportunity to carry out research directed at a more sustainable animal diet, primarily for tropical regions of Mexico like Campeche.

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