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Quantification of direct and indirect damage caused by *Diceroprocta bulgara* (Distant) (Hemiptera: Cicadidae) in lime

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Citas indirectas o paráfrasis: Cuando se interpretan o se comentan ideas que son tomadas de otro texto, o bien cuando se expresa el mismo contenido pero con diferente estructura sintáctica. En este caso se debe indicar el apellido del autor y el año de la referencia de donde se toman las ideas. Ejemplo:

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Geographical analysis of rice production and storage in Mexico, 1980–2018

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ABSTRACT

Objective: To analyze the production and storage of rice in Mexico, geographically and along 1980 to 2018; presenting cartography of the geographical location, rice storage centers, nodes, networks, and areas of influence generated by rice production.

Design/Methodology/Approach: The statistical database of "Apoyos y Servicios a la Comercialización Agropecuaria" (ASERCA; a resource database to support agricultural marketing) was used, as well as statistical information from the Mexico's information service "Servicio de Información Agroalimentaria y Pesquera, SIAP). Likewise, the cartographic basis for analysis was built in GIS. A vector database was digitized and integrated into a relational database for spatial analysis.

Results: Results of production, storage, distribution network and the rice consumption-storage forecast for Mexico in 2030 are presented. Using the statistical base, depicted in a GIS projection.

Limitations in the study: It is necessary to perform a spatial analysis per state to determine distance isochrons and to calculate planning scenarios.

Findings/Conclusions: The geographical analysis of rice production in Mexico maintains a territorial organization of cultivation, distribution, storage and consumption, which locates economic activities based on geographical factors of the primary sector, generating some geographical patterns for rice production, such as location, distribution and consumption per human settlement.

Keywords: geographic analysis, rice production, geographic information systems.

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INTRODUCTION

Rice (*Oryza sativa*) is one of the most consumed cereals in the world due to its nutritional benefits. It is considered that rice origin dates back more than 7 thousand years before the Christian era from India and China. The rice grain is the mature ovary and main product; when grain includes the husk, it is called Palay rice (DOF, 2017). The plant belongs to the grass family and to the genus *Oryza*. This genus includes several species; among which rice is very important. The *Indica* group grows in the tropical regions of India, Philippines, parts of the United States and Mexico (Parson, 1988).

Image by Tongpradit Charoenphon at Pixabay

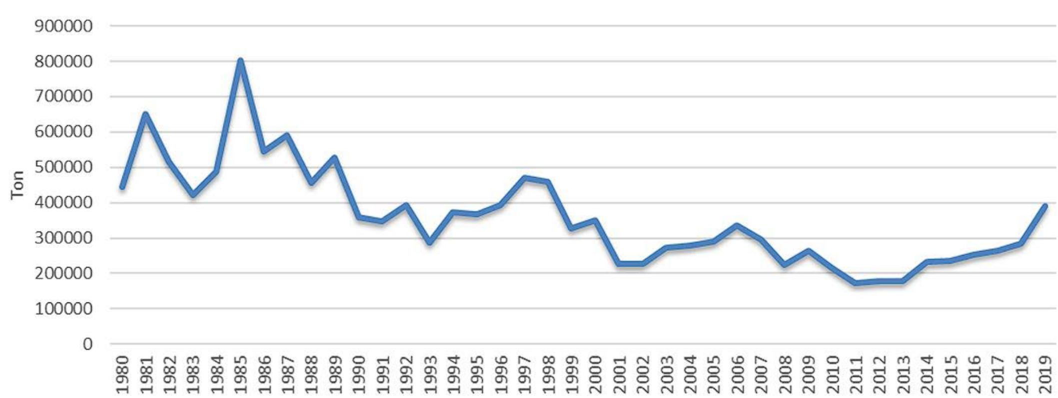
The rice grown in our country is cultivated under irrigation. Especially in central and northern areas of the country; rainfed cultivation areas are along the Mexican southeast. The main characteristic that rice-sowing territories must fulfill is a high content of organic matter that allow longer retention of water, which is essential to rice germination (Valverde, 2020).

Rice production in Mexico has shown variability from 1980 to 2019; also considering the various factors of agricultural activities and public policies that were modified during that period. All of them generated the national scope of rice production system; despite those, rice production in Mexico in 2019 was 390 118 tons (SIAP, 2019). We include below a graph of the historical rice production per year in Mexico.

Those states with geographical representation in the rice production in Mexico have an important value as economic spaces in the rural environment. These states are Campeche, Colima, Chiapas, Guerrero, Jalisco, Mexico, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Quintana Roo, San Luis Potosí, Sinaloa, Tabasco, Tamaulipas and Veracruz, which have historically been the producers of Mexican rice (Valverde, 2020).

Under the guidance of ASERCA (Apoyos y Servicios a la Comercialización Agropecuaria), in the storage program (for grains and oilseeds) "Capacidad Actual de Almacenamiento de Granos y Oleaginosas en México", facilities, locations, storage conditions, equipment and other characteristics that allow to issue a diagnosis on rice storage in Mexico should be accounted. The ambitious mission of this administration entity towards territorial management is strongly based on lands connotation. Therefore, it is geographically oriented, to know the location of the crops, to locate storage facilities and their operating conditions, plus the distribution routes of the grains and seeds of the country. Implementation allows to generate a cartographic sight of the connections needed for agriculture and food marketing.

The aims of the research are to geographically analyze the production and storage of rice in Mexico from 1980 to 2018. The mapping of nodes, networks and areas of influence generated by rice production is presented, which serve as an important input to understand those production and storage flows, generated by trade networks, that allow visualizing the potential distribution routes considering transit safety.



Graph 1. Annual Rice Production in Mexico (1980–2019).
Own elaboration bases on SIAP (2019).

MATERIALS AND METHODS

Statistical information

The statistics provided by the SIAP were integrated in a delimited temporary database classified from 1980 to 2018. This periodical consideration was dimensioned by the accessibility of the data provided by the agency, and a homogeneous database was structured and built for its analysis with the following data. (name; X-Y coordinates; Mexican state; municipality; type of warehouse; usage, storage capacity, product origin, distance to storage facility, destination, and distance from the storage facility to the destination).

The design of the survey on the relationship between the cultivation areas and the storage facilities was generated with the elaboration of a questionnaire, a scientific protocol that allows to gather complete information from a documentary point of view; to obtain a general and comprehensive description of the object of study; to narrow the problem capturing the relevant data; and to model the data in a object-implemented GIS database.

The questionnaire on capacity, infrastructure, equipment and characteristics of storage facilities, was applied at the national scale by the company Control y Tratamiento de Agroproductos S.A. de C.V. That company in collaboration with the association Apoyos y Servicios a la Comercialización Agropecuaria (ASERCA, 2013) set the objective of knowing the location, capacity, function and current storage conditions for grains and oilseeds in Mexico.

The applied questionnaire consisted of 53 questions divided into 5 modules that sought to document the situation in which more than two thousand storage warehouses interviewed operate. Within this large sample, geographic characteristics were obtained from the location of the storage facilities, in addition the knowledge of origin points and destination routes of the stored grains or oilseeds.

The GIS database created

With the assembled databases, we proceeded to digitize by means of polygonal vectors (Ortega, 2016) those rice-growing municipalities. Identifying with point vectors the storage warehouses and with linear vectors the roads fixing the flow route that connects cultivation areas and storage points. In addition, circular buffers were created with a diameter established by the distance (in kilometers) from the storage to the probable commercial destination, delimiting the areas of influence of the Mexican rice industry. This process allows to determine the impedance of the areas of influence that refer to the movement and type of resource flowing through the network, along with the type of geographic object and the direction of movement (Bosque, 2012).

The cartographic interface was carried out using QGIS v. 3.6.0 Noosa. Also, the database design was established with available information about the production development of rice. The data established by SIAP were used again as inputs in the geographic information model.

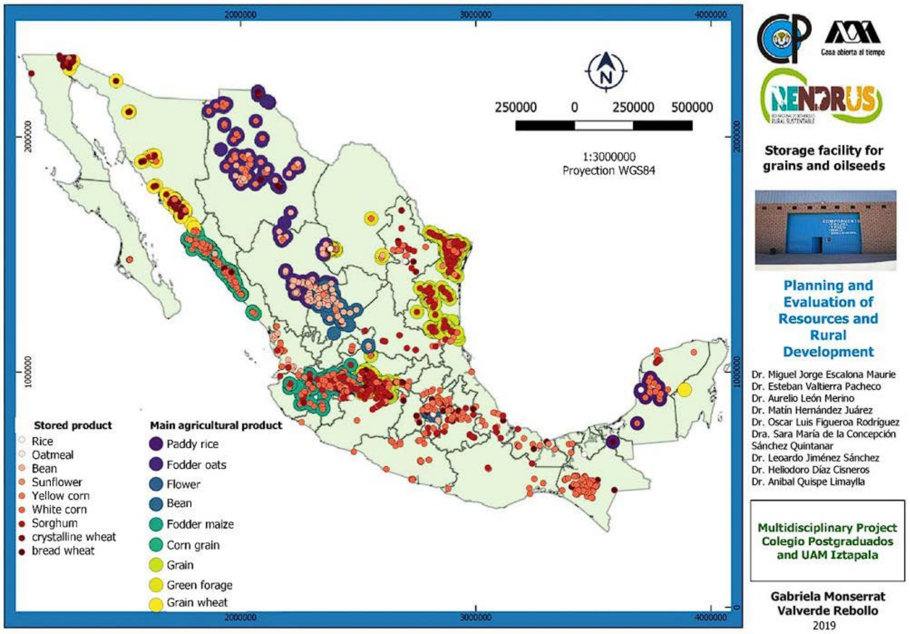
The spatial structure of the data starts from georeferencing the location of the Municipalities recorded with the presence of rice plantations from 1980 to 2018, based on the following records:

- Name of the rice-producing state
- Name of the rice-producing municipality
- Location (geographic coordinates)
- Hectares of Annual Cultivation
- Hectares of Annual Harvest
- Annual Production, in tons
- Productive Value, in MXN pesos
- Tons for annual consumption
- Consumption, in kg *per capita*
- Totals of National population census and intercensal population figures
- Presidential period
- Name of the President in the presidential period

The combined geographic, statistical and geometric data were classified according to their alphanumeric characteristic, in vectors represented as points, lines and polygons modeling their position and dimension values. This procedure was done through the use of GIS and cartographic products as complements of the scientific analysis of research (Buzai, 2011).

Once assembled the database of the total of 2,580 interviewed grain and seeds storekeepers, the first action was to concentrate the data of geographic relevance into maps. We determined to convert the coordinate system of each warehouse location represented in a sexagesimal system (degrees, minutes and seconds) to the metric units of UTM coordinates with the following formulas:

$$\text{Longitude} = ((\text{Hours}) + (\text{Minutes}/60) + (\text{Seconds}/3600)) * -1$$



Map 1. Spatial representation of the location of grain and oilseed storage facilities in Mexico.

Latitude=((Hours)+(Minutes/60)+(Seconds/3600))

The method of geographical analysis was based on a conceptual framework generated with studies of economic, agricultural and regional geography of Mexico, and a wide bibliographic consultation of documents such as Tratado de Geografía Humana (or Human Geography Framework), which laid the theoretical foundations of the research. (Hiernaux, 2006).

A total of 5 maps were obtained that allow visualizing the geographical scope of the economic, historical and cultural networks of rice production. Adding a little perspective of the proposed planning to improve the projective situation of rice consumption in Mexico.

RESULTS AND DISCUSSION

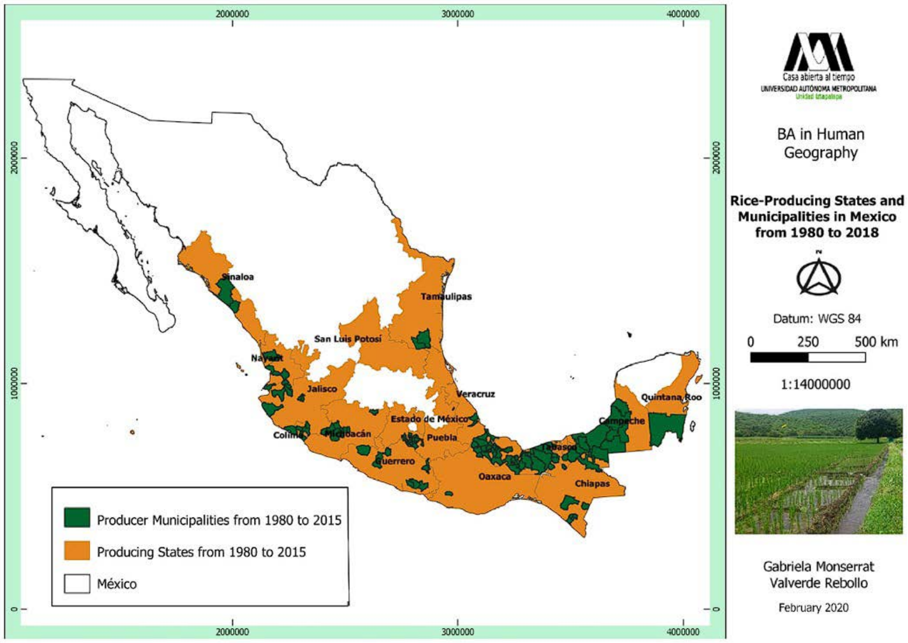
Rice Spatial Analysis from 1980 to 2018

Mexican producing states: The coverage that Mexico allocates for the rice production in 2018 was 17 out of the 32 Federation states allocating rice cultivation areas, according to the production surface records of the SIAP (2019).

The following map of rice producing municipalities and states in Mexico (1980 to 2018) shows that the southern part of the country is where to a greater extent the rice growing areas are located. However, the northern part of the country is where the highest production yield is obtained, especially in the state of Sinaloa.

It is remarkable the great coverage that Mexico allocates for the production of rice; until 2015, 17 out of Mexico's 32 Federation states are registered with rice production area.

Morelos has 14 rice-producing municipalities, followed by Veracruz with 8 and Chiapas with 7; Guerrero, Jalisco and Michoacán tie with 6 Municipalities dedicated to



Map 2. Rice producing municipalities and states (1980 to 2018).

rice production; Campeche and Nayarit have 5 Municipalities; Colima And Tabasco with 3 Municipalities; the state of Mexico 2, like Oaxaca; Sinaloa, Tamaulipas and Quintana Roo are leveled with 1 rice-producing municipality.

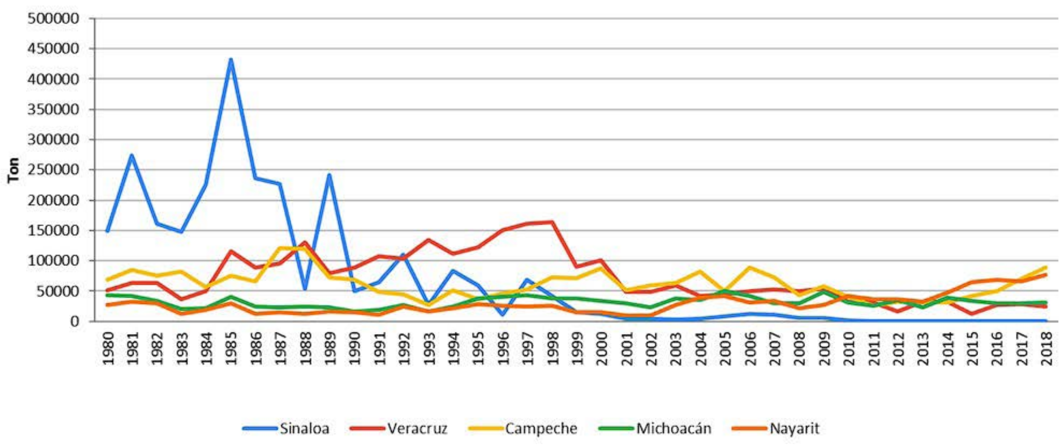
In 1980 there was a total area of 106,541 hectares of rice cultivation; with the exit of San Luis Potosí, the cultivation area was reduced to 96,838 ha in 1992; and in 2002 total rice production area was 54,982 ha given the null production of Puebla. In 2011, rice sowing area was further reduced to 36,811.44 ha, due to the loss of Sinaloa, which was rebounding as a producer state.

Former positioning changed among the states; to the South by Campeche, Tabasco and Chiapas, as to the North by Tamaulipas. As well as to the East by Veracruz and to the West by Nayarit, Jalisco, Colima, Michoacán and Guerrero, plus the central region made up of the sates of Mexico and Morelos. That production dynamics reflects extension of the territory suitable for rice agriculture.

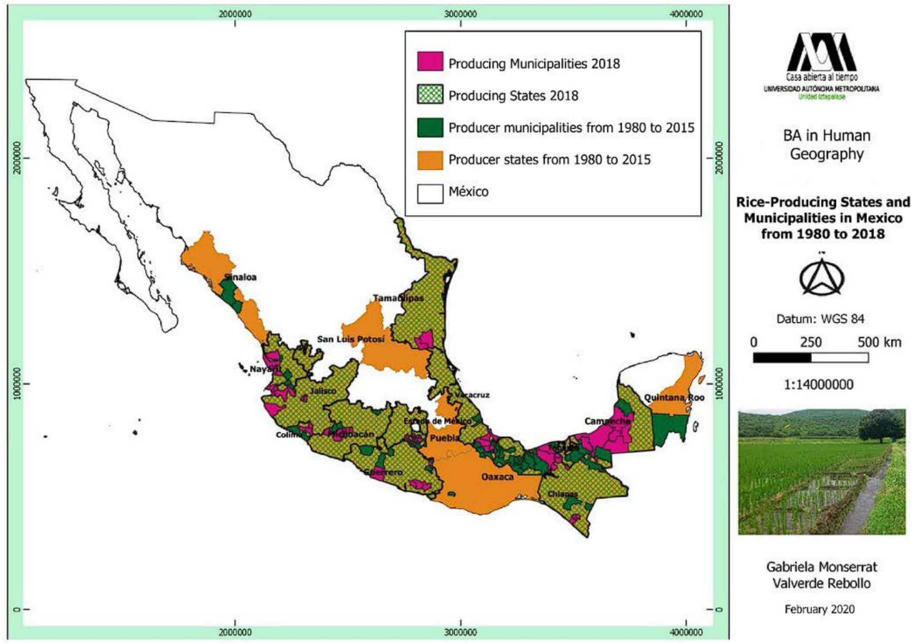
If the 5 years of study presented in the Atlas Agroalimentario (2012–2017) is extended to 38 years (1980 to 2018), the positions of rice-producing states change very little. Veracruz, Campeche, Michoacán and Nayarit remain in the ranking of 5 municipalities. The only significant change is that from 1980 to 2018 Sinaloa was present and with valuable contributions in terms of production. In contrast with the period from 2012 to 2017 when Colima appeared, but with fewer elements of cultivation and harvest areas as well as rice tons produced annually. For this reason, it appears of utmost importance to regain the position of the productive performance that Sinaloa represented for the Mexican rice industry.

The timely comparison among the states and municipalities producing rice between 1980 and 2018 shows the negative loss of five rice-producing states, from the South (Quintana Roo) and Southwest (Oaxaca), across the Center (Puebla), to the Northeast (San Luis Potosí) and Northwest (Sinaloa).

Geographical location of the infrastructure, equipment and characteristics of storage facilities. The diagnosis obtained from the responses to the questionnaire allows a deeper approach to the key characteristics with which warehouses operate to keep the grains



Graph 2. Main rice producing states in Mexico (1980 to 2018).
Own elaboration bases on SIAP (2019).



Map 3. Map of rice producing states and municipalities.

of white and yellow corn, beans, bread wheat, crystalline wheat, rice, sorghum, oats, barley and rye; along with the seeds, chickpea, soybean (and soybean paste), safflower, sunflower, sesame, canola (turnip seed), cottonseed, among others. In addition to identifying their conservation and the routes of transfer for distribution.

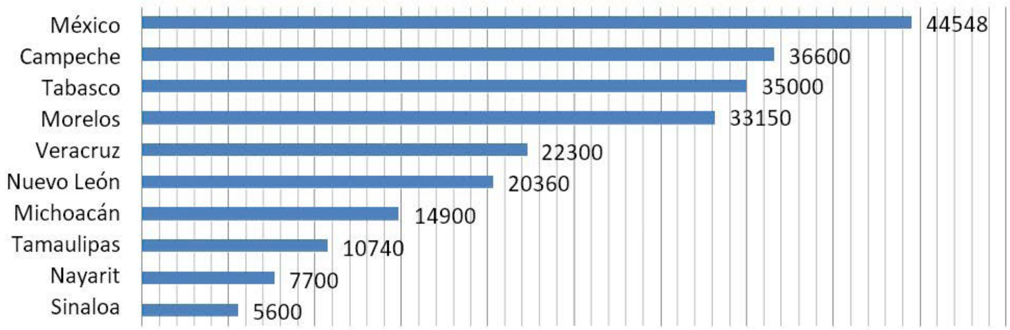
The databases obtained from applying the questionnaire (ASERCA, 2013), allow us to find the tonnage capacity to safeguard the commercialized seeds. We found a total of 2,580 storage locations, divided into 2,118 active and 462 inactive. Out of the 2,118 active warehouses, 17 rice processing facilities were identified in the country, in 10 states of the Republic.

The largest storage capacity is in the state of Mexico (44,548 tons). At La Paz municipality, the rice stored there comes from crops of Veracruz, transferred by rail from Veracruz port to the rice-processing facilities in the state of Mexico, where the product is allocated for commercialization within that state territory.

Campeche has two rice-processing facilities, one in the municipality of Campeche and the other in Hecelchakan, with a joint capacity of 36,600 tons. Those facilities receive Palay rice from the same municipalities and from Quintana Roo, in order to distribute that type of rice to another state in the North.

Tabasco, in the municipality of Teapa, has a hub and distribution center, which means that Teapa receives, stores and distributes the grains. That facility has a capacity for 35,000 tons of rice, grown in the same state and sold to another state in the north.

Rice production, storage and distribution network. The analysis of networks and flows in geography has become an essential methodological path to understand the unity of the world and the diversity of places. The study of social networks has diverse backgrounds and 67 paths for geographic research. On the one hand, there are references to the mainly



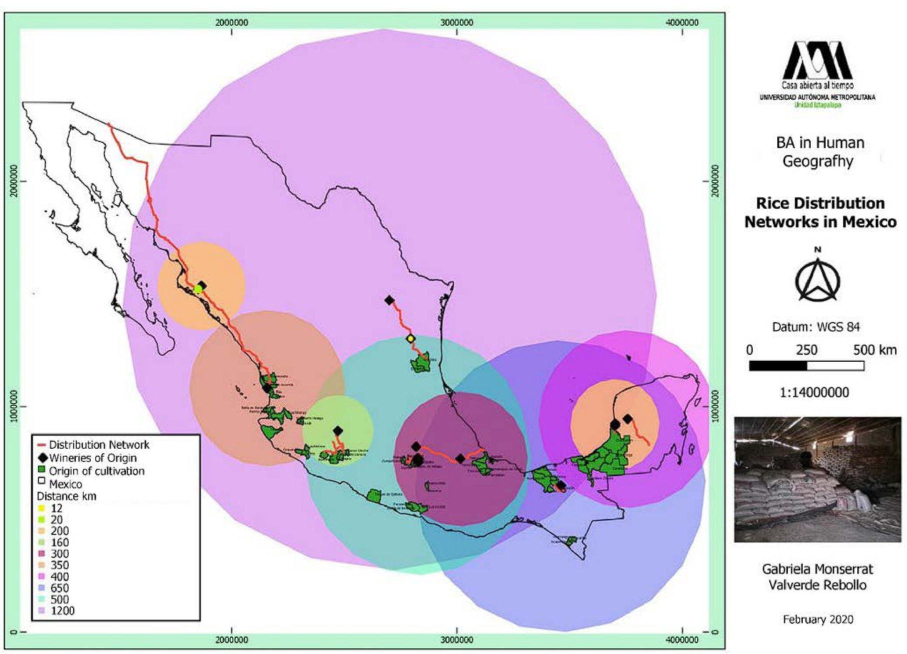
Graph 3. Capacity (tons) of rice storage per state.
Own elaboration bases on SIAP (2019).

material characteristics of their assets, which is expressed for example in the organization of transport (Rosales, 2010).

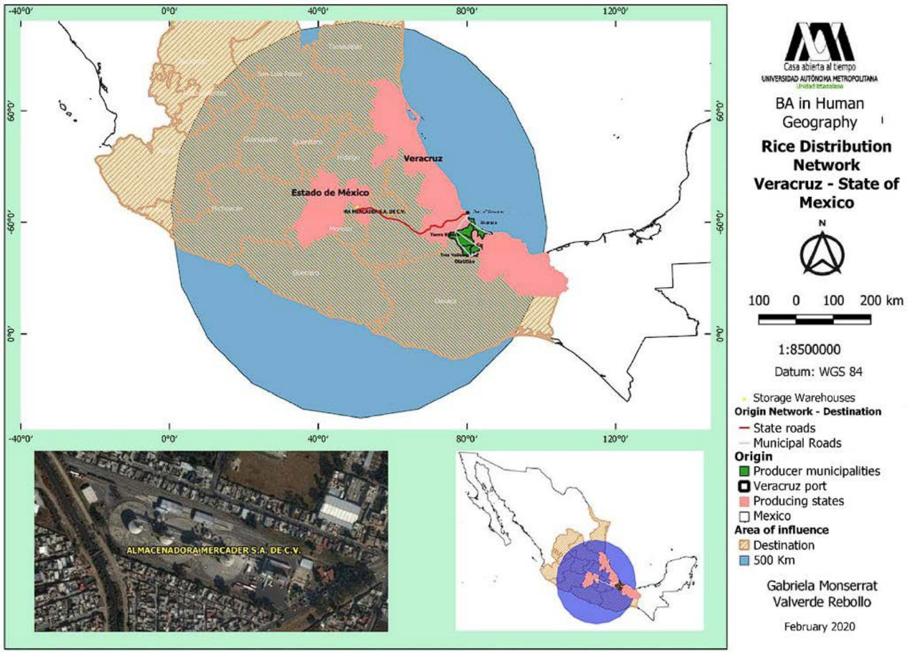
The rice distribution networks in Mexico are strongly marked on the territory, the sowing surface shows the potential area for the commercial development of the grains that after their harvest are taken to the warehouses that process the product. Then, with the rice packed for sale, it is distributed to the most populated places and to those territories where rice cultivation does not exist.

Both crop lands and warehouses are fixed production points, therefore locatable. Roads or railways form the commercial connection networks which generate an area of economic influence.

Monetary flows circulate in the opposite direction to material flows. Information flows are inscribed in all directions and are reciprocal (Claval, 1980).



Map 4. Rice distribution network in Mexico.



Map 5. Rice distribution network Veracruz-Estado de México.

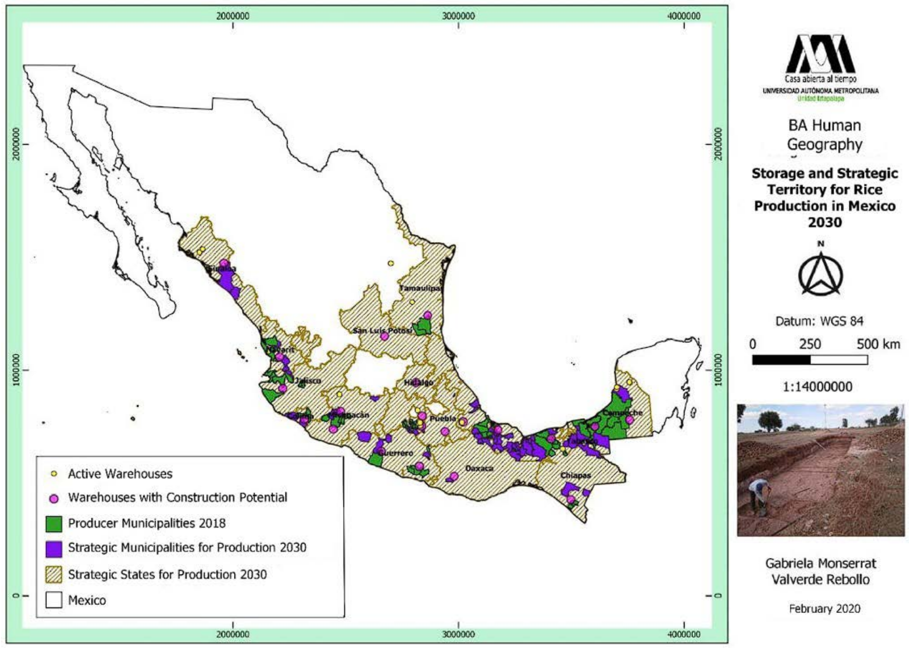
Undoubtedly, this cartographic activity has shown the importance of the geographic foundation in the study of the diverse rural expressions in the country, which could widen the path for the institutionalization of rural geography in Mexico (Ávila, 2010). The disposition of activities around the markets where flows converge and where decisions are readjusted and clarified (Claval, 1980) is proposed.

The analysis of the various forms of agricultural activity occupies one of the most prominent places in geo-economic studies, not only because it represents a great variety of crops and working conditions, but because, on the one hand, agriculture is a very accurate exponent from the influence of natural and social factors and, on the other hand, despite the advances registered in the industrialization of large areas of the planet, this work is still the most important of all that is carried out in most nations (Bassols, 1984).

Mexico's National Planning 2017-2030. The concern of the Mexican government to achieve food self-sufficiency in the rice sector has remained constant and visible. Since 2016, a study was carried out to plan the future of rice-production in the country, resulting in the "Planeación Agrícola Nacional 2017–2030 for Mexican Rice".

With the strategies proposed for 2030 and seeking to achieve Mexico's domestic self-sufficiency, it is necessary to at least quadruple annual rice production. This means expanding the number of hectares for sowing and also protecting soil resources through the care of harvesting techniques.

The challenges that Mexico has to face in order to achieve productive self-sufficiency are extensive and are even more dimensioned when considering the National territory extent. Consumption is a factor for growth, just like the population. Mexico should be prepared to increase national productivity, as well as increasing the value of rice grain paid to producers; tough at the same time it should seek to favor local consumption by inhabitants of the rice-producing municipalities.



Map 6. Strategic storage for rice production in Mexico (2030 scenario).

CONCLUSIONS

Rice is for the world, a basic good that satisfies one of the inherent needs of human beings. Rice production and consumption contribute 20% of the food energy of the planet due to its nutritional value. Because of this, it is of paramount importance to care for the terrestrial surface containing the favorable environmental elements for rice cultivation, between the latitudes 23° S and 23° N. These areas are under tropical climates with warm temperature and rains in summer; mainly located in coastal areas that configure a favorable territory for agricultural use.

Mexico has the geographical, hydric, social and cultural conditions to increase rice production, considering as a limit the 23° N parallel within the Tropic of Cancer, across (from East to West) the states of Tamaulipas, San Luis Potosí, Zacatecas, Durango and Sinaloa. The limit to the South is the state of Quintana Roo; thus, so for that state we recommend planning the reactivation of rice production.

The Mexican government should consider the increase in the area under rice cultivation. As well as that increase should respect the established agricultural land-use frontiers and the geographical and ecological characteristics of those areas adequate for sowing and harvesting rice.

The periodic review of grain storage locations can influence the search and implementation of technological innovations that maintain optimal environmental conditions to keep rice grain safe. At facilities with low temperatures, long periods of aeration, and low relative humidity; primarily considering that storage factors are completely opposite to the characteristics necessary for sowing seeds and growing rice plants.

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Quantification of direct and indirect damage caused by *Diceroprocta bulgara* (Distant) (Hemiptera: Cicadidae) in lime

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ABSTRACT
Objective: To quantify the direct and indirect damage caused by *Diceroprocta bulgara* in lime cultivars: Persian, Mexican with and without thorns, Colimex and Lise; on the Coast of Oaxaca, Mexico.
Design/Methodology/Approach: The damage caused by *D. bulgara* was calculated by counting nests in trees of five lime cultivars (Persian, Mexican with thorns, Mexican without thorns, Colimex and Lise). In each plot, 15 trees were selected randomly to evaluate the variables: height, crown diameter, damaged growth flows, damaged growth diameter, damage length, and number of eggs. The loss of fruit from the indirect effect (kg ha^{-1}), was estimated in three densities of Colimex and Lise.
Results: The damage ranged between 78.6% (Mexican without thorns) and 94% (Colimex). The trees of largest size showed a higher percentage of affected flows. The second flow showed the highest frequency of damage (32%), while the lowest frequency was obtained in the first flow (17.3%). The lowest number of eggs was 26 in flow one in Mexican lime without thorns, while the highest was 171 in Mexican lime without thorns. The greatest number of eggs was observed in Persian lime (371). A significant relationship was determined between the number of eggs and the damage length and flow diameter (<0.05). The highest number of mummified fruits (15.6) was observed in Colimex (density of 312 trees ha^{-1}) and the estimation that up to 146 kg of fruit could be lost.
Findings/Conclusions: This study quantifies for the first time the direct and indirect damage caused by oviposition of the cicada *D. bulgara*, which represents a loss of fruit in productive trees. Studies on the insect's biology are suggested for integral management plans.

Keywords: Citruses, fruit loss, cicada.

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INTRODUCTION

Mexico was the main producer of lime in the world during the production cycle 2018-2019, with a production volume of 2.6 million tons, corresponding to 34% of global production (USDA/FAS, 2020). Two species of lime are cultivated in Mexico: *Citrus aurantifolia* (Christm.)

Swingle, and *Citrus latifolia* Tanaka ex Q. Jiménez; the first is distributed primarily in the Pacific watershed and the second in the Gulf of Mexico (Orozco-Santos *et al.*, 2013). In Oaxaca, Mexico, the Central Valleys Experimental Field, Coast of Oaxaca Experimental Site of the National Institute for Forestry, Agriculture and Livestock Research (*Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias*, INIFAP), has evaluated varieties of lime that adapt to the agroecological conditions of the Coast region, which allow diversifying the alternatives for production and commercialization in international markets (Ovando-Cruz *et al.*, 2015).

The main insect pests reported for the lime crop in Mexico are: psyllid (*Diaphorina citri* Kuwayama), leaf miner (*Phyllocnistis citrella* Stainton), white fly (*Dialeurodes* sp.), blackfly (*Aleurocanthus woglumi* (Ashby)) and some species of aphids (*Aphis spiraecola* Patch and *Toxoptera aurantii* Boyer) (Grafton-Cardwell *et al.*, 2013; Ovando-Cruz *et al.*, 2015).

Orozco-Santos *et al.* (2014) reported the presence and possible damage associated to a cicada (Hemiptera: Cicadidae) in lime plants in Colima, México. Then, Sánchez-García *et al.* (2019) identified this species as *Diceroprocta bulgara* (Distant) and reported various citruses as hosts for oviposition. During this process, females cause longitudinal lesions on the bark of the trees' apical branches, which later leads to their death.

This study quantified the direct and indirect damage caused by oviposition of *D. bulgara* in cultivars of Persian lime and Mexican lime with and without thorns, as well as in the INIFAP varieties Colimex and Lise; the study also discusses the implications of damage caused by the insect and possible alternatives for control.

MATERIALS AND METHODS

Study area

The study was conducted in two plots with lime of INIFAP's Coast of Oaxaca Experimental Site, located in Río Grande, Villa de Tututepec, Oaxaca, Mexico. The first plot with one-year-old plants of the cultivars: Mexican with and without thorns, and Persian. The plants were established under a design with strips with distances of 4 m between plants and 8 m between lines. The second plot with three-year-old plants of the varieties Colimex and Lise, established in a system of strips with distances of 8×4 m, 7×4 m and 6×4 m.

Evaluation of the damage caused by cicadas

The evaluation was performed in plants from the two plots selected. In the first place, the presence of damage from *D. bulgara* (nests) was found in each tree of the five citrus cultivars. Then, 15 trees were selected randomly in each plot, of which data were taken of tree height and crown diameter; the number of flows with damaged vegetative growth from the cicada and the number of terminal branches affected in each flow were quantified. The diameter of the vegetative flow and the length of the damage were also measured in each of the samples, and the eggs laid were counted. The presence of at least one oviposition zone in one of the last three growth flows was considered as a characteristic to consider a damaged flow. The severity of the attack from cicadas was classified according to the number of flows with oviposition, and eight damage scales were proposed for that purpose:

Class 1=without damage, Class 2=1 to 5 flows, Class 3=6 to 10, Class 4=11 to 15, Class 5=16 to 20, Class 6=21 to 25, Class 7=26 to 30, and Class 8=more than 30.

The number of fruits affected indirectly by *D. bulgara* was recorded in the plants in productive stage, and an estimation of loss of fruit was conducted (kg ha^{-1}) based on the plantation density.

Statistical analysis

The variables stem diameter, damage length and number of eggs were analyzed through a multiple linear regression model with the software SAS version 10.0. This analysis was confirmed with tolerance (T) higher than 0.10, as variance inflation factor (VIF) lower than 5%, and the significant value of F and validity of the model by the lowest value of the Akaike information criterion (AIC).

RESULTS AND DISCUSSION

Oviposition by *D. bulgara* was observed in the two experimental plots. The process of oviposition generated partial damage in the vascular bundles of the affected shoots, which led to the death of the vegetative flow. This process was previously reported by Sánchez-García *et al.* (2019). The period of oviposition coincided with the phenological phases of flowering and fructification of the plots, which indirectly caused the death of small flowers and fruits close to harvest (Figures 1 and 2).

Table 1 presents the percentage of incidence of citrus trees affected by D. bulgara. The incidence of damage was quantified from 78.6 to 94%, based on the number of trees from each cultivar.

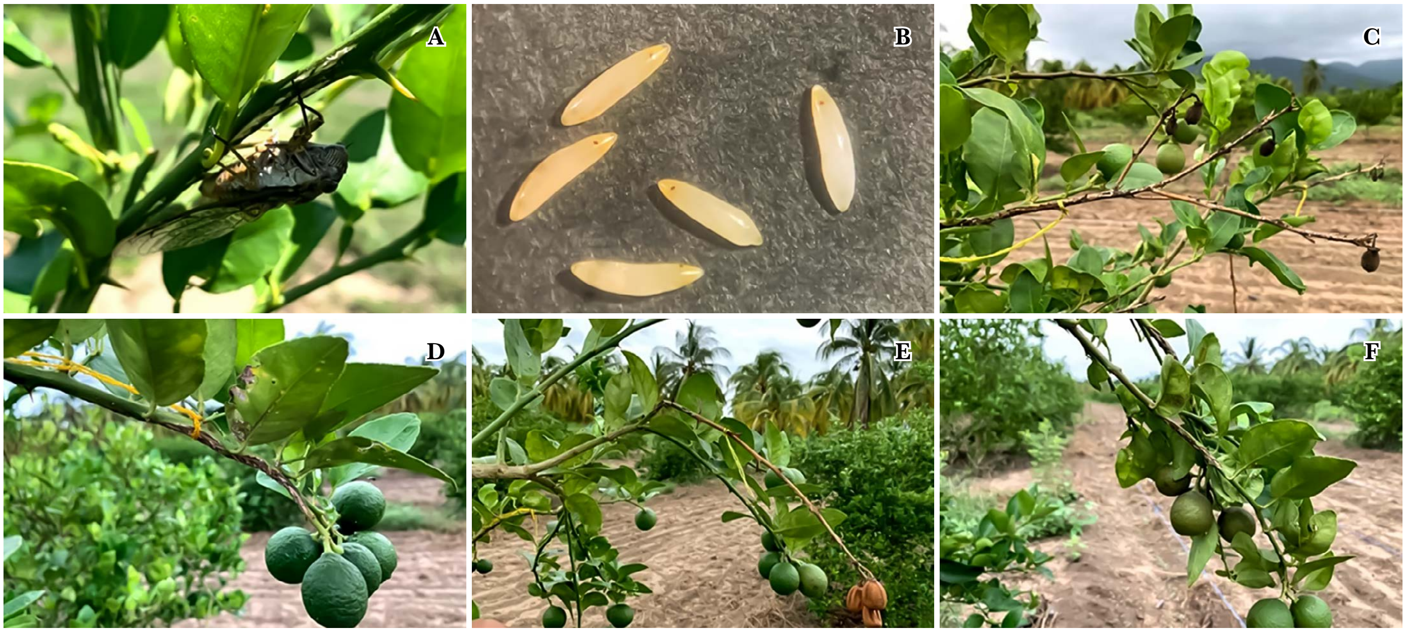

Figure 1. Female cicada of *D. bulgara* in Mexican lime plant (A), eggs (B) and damage caused in terminal flows and lime fruits as result of the oviposition of eggs (C-F). Río Grande, Villa de Tututepec, Oaxaca. June 2020.

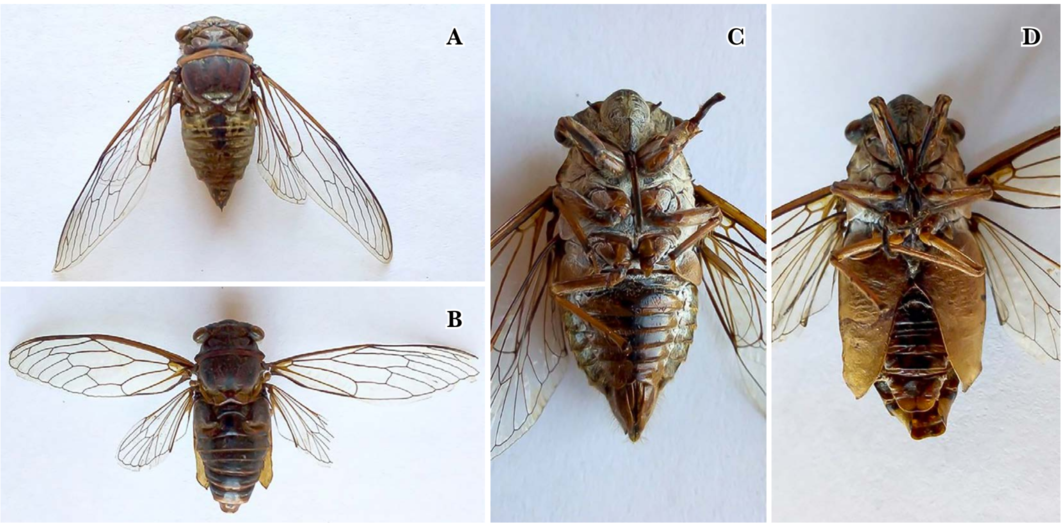

Figure 2. General aspect of the male and the female of *D. bulgara*. Female (A and C); male (B and D).

Table 1. Incidence of the damage by *D. bulgara* in trees from five citrus cultivars.

Cultivar	Number of tres (n)	Trees with damage (%)
Persa lime	178	83.70
Mexican with thorns	224	83.03
Mexican without thorns	224	78.57
Colimex	320	94.06
Lise	286	90.21

Regarding the relationship between the size and diameter of the trees with the average of damaged flows, a low correlation was observed where larger plants showed a higher percentage of affected shoots (Table 2). This can be attributed to the cicada females having a greater area or leaf cover to carry out oviposition compared to smaller plants. On the other hand, the average of affected terminal branches fluctuated negatively in terms of size of the trees (Table 2).

Table 2. Height and crown diameter and terminal growth flows (1-3) of lime trees with damage by *D. bulgara*.

Cultivar	Plant height (cm)	Crown diameter (cm)	Average number of damaged flows	Average number of affected terminal branches
Persa lime	122.40	72.73	2.33	4.73
Mexican with thorns	124.26	94.50	2.35	4.11
Mexican without thorns	137.00	70.83	2.06	3.40
Colimex	171.68	201.83	4.30	3.73
Lise	192.57	255.00	3.40	3.56

In the five citrus cultivars evaluated, damage by the cicada was observed in the three terminal flows. The flow with highest damage frequency was flow 2 (average of 32%) and the one with lowest frequency was flow 1 (average of 17.3%) (Table 3).

The average number of eggs laid per each flow evaluated was variable for each citrus variety, with a minimum of 26 for flow 1 in the cultivar Mexican without thorns, and a maximum of 171 in the same cultivar (Table 4). In general, a higher number of eggs was counted in Persian lime (371 eggs), compared to the Mexican lime with thorns (245 eggs). These values are higher than those reported by Sánchez-García *et al.* (2019), who counted an average of 48.4 eggs in Mexican lime.

The criteria used in the statistical analysis by multiple linear regression were accepted, based on the assumption of non-collinearity between the variables, through the criteria of tolerance values (T>0.10) and the variance inflation factor (VIF<5.0) (Table 5). Likewise, in the analysis of variance, the statistic F was highly significant (<0.05), which indicates that there was significant linear relationship of the dependent variable number of eggs and the variables damage length and flow diameter.

Table 3. Frequency of oviposition by *D. bulgara* in the last three growth flows in five lime cultivars.

Cultivar	Samples (number)	Flow 1	Flow 2	Flow 3
Persa lime	15	15.55	28.88	20.00
Mexican with thorns	15	20.00	33.33	33.33
Mexican without thorns	15	17.77	33.33	33.33
Colimex	15	20.00	31.11	33.33
Lise	15	13.33	33.33	31.11

Table 4. Damage length, flow diameter and eggs per growth flow in five lime cultivars.

Cultivar	Flow 1			Flow 2			Flow 3			TH
	L	D	H	L	D	H	L	D	H	
Persa lime	10.0	1.9	82.8	11.4	2.8	128.4	10.6	4.4	160.6	371.8
Mexican with thorns	3.3	1.5	26.7	8.8	2.5	143.0	8.1	3.9	171.0	340.7
Mexican without thorns	3.9	1.7	36.7	7.5	2.9	102.8	5.8	4.1	106.0	245.5
Colimex	6.1	1.8	38.8	10.4	2.8	137.7	10.8	4.2	158.2	334.7
Lise	7.4	1.8	53.5	8.6	2.8	88.0	7.6	3.3	127.0	268.5

L=Length of flow damage, cm; D=diameter of flow, mm; H=Eggs per flow, TH=Total of eggs in flows 1-3.

Table 5. Multiple linear regression analysis of the number of eggs (E) of *D. bulgara* in function of the variable damage length in the different flows in the five lime cultivars (FL) and the respective flow diameter (FD).

Variable	parameter			R^2	R^2 adjusted	EMS	AIC	tolerance >0.10		VIF <4.0	
	β_0	β_1	β_2								
DL y FD	-55.5**	11.4**	24.7**	0.58	0.57	3100.3	1433.9	0.9	0.9	1.0	1.0
Modelo				Huevecillos=−55.569+11.437*LD+24.75*DF							

EMS=Error mean square; LD=Damage length; FD=flow diameter; VIF=Variance inflation factor.

The number of fruits damaged indirectly by the effect of oviposition of the cicadas was variable between the lime cultivars evaluated and the density established. The largest number of mummified fruits (15.6) was observed in trees of the variety Colimex in productive stage (density of 312 trees ha^{-1}); while the lowest number was observed in the variety Lise in density of 312 trees ha^{-1} (5.25 mummified fruits) (Table 6). Data from the estimation of fruit loss suggest that up to 146 kg ha^{-1} could be lost in the variety Colimex from the indirect effect of oviposition by the cicada (Table 6).

Previous studies have reported that different species of cicadas affect different plant species. In Canada, the cicadas of long cycle of 13 to 17 years (*Magicicada* spp.) cause damage on the apical branches of young oaks, provoking a reduction of 30% of total biomass (Koenig and Liebhold, 2003). In Spain, cicadas of the genus *Cicada* sp. are considered secondary pests of olive trees, since they affect the apical branches of young plants and the nymphs feed off the roots (González *et al.*, 1998). Logan *et al.* (2014) report that the cicada *Amphipsalta zelandica* (Boisduval) in New Zealand rose as an emergent pest in kiwi crops since the year 2000, causing the same type of effects in branches and roots. On the other hand, in Brazil, various studies have been conducted of the damage caused by the common giant cicada *Quesada gigas* (Olivier), which is considered to be one of the main pests in coffee, found in 87% of the total cover of coffee plantations (Kubota, 2013).

The damage caused by cicadas happens in two different phases during the life cycle of insects. In their first stages, nymphs feed off the sap that they extract from the roots; then, in their adult stage, females cause lesions on the youngest branches during oviposition, and these branches later wither and die (Zanuncio et al., 2004; Sánchez-García et al., 2019). In this sense, the correct taxonomic identification of the insect, as well as the recognition of the duration of the life cycle are two important factors to develop an integral management plan (Kubota, 2013; Logan *et al.*, 2014). No control methods are reported in Mexico for this insect, although various authors have suggested some alternatives for its management. González *et al.* (1998) recommend two alternatives for the control of *Cicada* sp. in olive crops: the use of natural enemies such as the mite *Pyemotes ventricosus* (Newport) which has a depredation rate of 82% on first-instar nymphs of cicadas; and the use of lure branches or sticks such as sunflower stems or soft wood, with the aim of promoting oviposition in these sites and not in the crop of interest. In the case of damage caused by *Q. gigas* in coffee, the use of a plastic filling or plant cover traps has been recommended, with the aim of breaking

Table 6. Number of lime fruits damaged indirectly by oviposition of *D. bulgara* and estimation of fruit loss in kg ha^{-1}.

Cultivar	Distance (m)	mummified fruits (number)	Loss of fruits (kg ha^{-1})
Colimex	8×4	15.60	146.25
	7×4	13.85	129.40
	6×4	10.90	142.11
Lise	8×4	5.25	54.22
	7×4	6.43	69.03
	6×4	6.68	70.69

the life cycle, whether by preventing nymphs from reaching the ground or avoiding adults from performing the nuptial flight, in a similar way as other insect pests that need the ground to complete their cycle (Zanuncio *et al.*, 2004; Coria-Ávalos and Ayala-Sánchez, 2010).

On the other hand, it has been mentioned that the promotion of monocrop has favored the excessive growth of pests or the emergence of secondary pests that had not been reported before. This phenomenon is common in insects with great ability to move through the air and with a massive reproduction strategy, as is the case of cicadas (Reis *et al.*, 2002). Presently, there are few cases reported of secondary or later invasions from a species of cicada on a new host with grave repercussions. In 2002, in Brazil, an infestation of *Q. gigas* was reported in a reforested area only with trees of the species *Schizolobium amazonicum* (Huber), affecting 15 ha (Zanuncio *et al.*, 2004). Sánchez-García *et al.* (2019) reported for the first time the lime *C. aurantifolia*, as well as other citrus species and wild plants, as hosts of *D. bulgara* in Mexico. These new records could represent new cases of colonization of different hosts by cicadas present in the country; however, various studies about the biology of the cicada are still necessary.

CONCLUSIONS

In this study, the direct damage caused by the oviposition process of cicadas on the terminal branches of five lime cultivars was quantified. The results show the quantification of the indirect damage by the insect through the estimation of the fruits lost during oviposition; thus, future studies could deduce the economic loss caused by this insect. Finally, to be able to establish a program for Integral Pest Management (IPM) focused on *D. bulgara* in citruses, it is necessary to understand first the biological cycle, then to estimate the damage caused, and based on this, to develop an adequate control plan for citruses in the region of the Coast of Oaxaca.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Microbial biomass and earthworms as indicators of soil quality under contrasting management practices in small-scale dairy systems

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ABSTRACT

Objetive: To determine soil quality under two contrasting agricultural management practices, based on microbial biomass and earthworm density, as compared to untilled control soils in two seasons (dry and wet), in different production units of small-scale milk production systems.

Methodology: The work was conducted in ten production units in the municipality of Aculco, Estado de México, Mexico. We analyzed physical, chemical, and biological soil indicators (microbial biomass and earthworm density). We conducted an ANOVA with a 3×2 factorial arrangement (three systems [maize, grassland, and control] and two seasons [dry and wet]).

Results: Values for all quality indicators in maize-cultivated soils were low, but increased in the wet season. Parameters in pasture-cultivated soils were similar to control soils.

Implications: These results determine the conditions of the soils used in milk production systems.

Conclusions: Some of the parameters assessed can be used as indicators of soil degradation and to strengthen other indicators which lead to an improved assessment of these systems' sustainability.

Key words: Agricultural management practice, Soil quality, Small-scale milk production systems.

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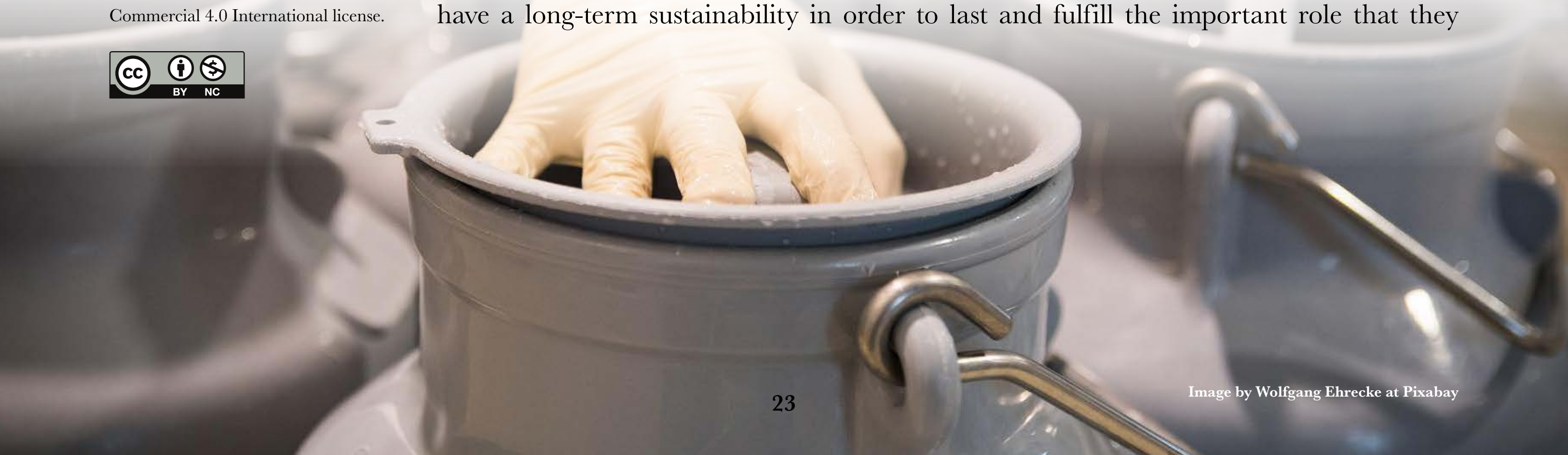
INTRODUCTION

In the context of agricultural production, small-scale dairy systems (SSDS) play an important role worldwide as an option to relieve poverty and improve quality of life in rural environments (McDermott *et al.*, 2010). These systems comprise production units with small farming land areas and herds of 3 to 35 cows (plus their replacements). They basically depend on family workforce and sell milk as a source of income. SSDS should have a long-term sustainability in order to last and fulfill the important role that they



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play within communities (Fadul-Pacheco *et al.*, 2013). Consequently, Mexico and the rest of the world have posed the need to reconcile the preservation of natural resources with agricultural production in small-scale dairy systems, particularly as a result of the importance of nutrient management in agricultural activities, which has a direct impact on soil quality (Gourley *et al.*, 2012).

Soil is a living, heterogeneous, and dynamic system that includes physical, chemical, and biological components, as well as their interactions. Each soil function includes or is the result of the interaction of its different physical, chemical, and biological properties, which can serve as quality indicators, as long as they can be measured qualitatively or quantitatively and propose an idea about soil functioning (Navarrete *et al.*, 2011). When assessing soil quality, different management systems can be compared in order to determine their respective effects on edaphic quality. Likewise, taking measurements in the same area throughout time can help to monitor soil quality trends—which are determined by soil use and management—, to compare problem areas within a piece of land with non-problem areas, and to compare measured values with edaphic benchmarks or with the natural ecosystem (Lemaire *et al.*, 2014). Soil quality indicators are seen as measuring tools that must provide information about properties, processes, and their characteristics (Pascual-Córdova *et al.*, 2017).

Soil deterioration is a natural process that can be reverted by adding pastures, which can restore some aspects of soil fertility (organic matter and biological properties), but it can also lead to content decline of several edaphic nutrients. This deterioration is difficult to perceive, because crops cultivated on a previously pastured soil usually present a better growth. Pasture requires nitrogenous and phosphorous fertilization, which allows the soil to recover the macronutrients removed during production (Abbona *et al.*, 2016). Determining the prevailing condition of soil in the SSDS of the municipality of Aculco, Estado de México, Mexico, will help to lay the foundations to improve management practices that increase the ecosystem's sustainability.

The objective of this study was to determine the quality of two soils under contrasting agricultural management practices, as compared to a control soil, using microbial biomass and earthworm density as indicators. The study was carried out during the dry and rainy seasons in ten different small-scale milk production units.

MATERIALS AND METHODS

Study area

The work was done in the municipality of Aculco, Estado de México, Mexico. The municipality is located at an average altitude of 2,440 m; the climate is warm sub-humid, with summer rains reaching a 700-1,000 mm precipitation (INAFED, 2015).

Production units and agricultural management

The work was carried out in ten production units. The selection criterion was at least 10 uninterrupted years of managing 1-2 ha plots where maize and pasture for cutting were cultivated together.

The agronomic management of the plots starts in February and March (plowing (fallowing), harrowing, and irrigation), followed by sowing in April. A first and a second weedings are carried out one and two months after sowing, respectively. These activities are mechanized through the use of tractors, plows, disc harrows, seeders, and croppers. Maize plots only receive a first irrigation, which allows farmers to sow in April, before the rainy season begins. The crops of choice are white maize, due to its better yield, and native maize, which is selected by producers themselves. Improved or open-pollinated varieties are also used (Aculco, Niebla, and Aspros).

Plots where grassland is cultivated have limited access to irrigation: they are only watered every four weeks, during the dry season. Grasslands are sown with different kinds of grasses, among which ryegrass (*Lolium multiflorum* L.) and white clover (*Trifolium repens* L.) are the dominant species. Traditionally, grasslands in the study area are managed based on a cut and carry system.

Unmodified zones in each farm were selected as control areas. Their soils had not been disturbed for at least 40 years and therefore were rich in native plants.

Soil sampling

One soil sample per production unit was taken each season: between February and March for the dry season and between June and July for the rainy season. Since the plots were irregular, each plot was divided into four subplots, where a stratified random sampling was conducted. A compound sample made up of 10 subsamples was collected in each subplot, adding up to four compound samples per plot.

Samples were taken at a depth of 0-20 cm and subsequently transported to the soil laboratory of the Instituto de Ciencias Agropecuarias y Rurales (ICAR), where they were dried at room temperature in the shade (18±2 °C), grinded, homogenized, and sieved through a 2-mm sieve.

Physical, chemical, and biological soil analyses

Based on the Mexican Official Standard NOM-021-RECNAT-2000, the total nitrogen content (NT) was determined using the Kjeldahl method, and the organic matter (OM) and total organic carbon (TOC) content with the Walkley-Black method. Gravimetry was used to determine moisture content, the graduated cylinder method was used to establish bulk density, and an electronic potentiometer was used to record the pH.

Determination of microbial biomass

The fumigation-incubation method (Jenkinson *et al.*, 2004) —the quantification of the CO_2 produced by soil samples— was used to determine the soil's microbial biomass carbon (MBC).

Determination of earthworm population

Earthworm population was determined following the Anderson and Ingram method (1993). The sampled monoliths were manually examined to collect earthworms and place them in glass jars with a 4% formaldehyde solution (Brito-Vega *et al.*, 2006).

Statistical analysis

The experimental design was a 3×2 factorial arrangement that considered the three systems (maize, grassland, and control) and both seasons (dry and rainy). The results were subjected to a variance analysis according to the following model (Kaps and Lamberson, 2004):

$$Y=\mu+M_i+E_j+M^*E_{ji}+R_k+e_{ijkl}$$

Where: μ: mean value; M=effect of the system (i=1, 2, 3); E: effect by season (j=1, 2); R: repetitions (producers) (k=1,…, 10); e=residual term. When differences were found between treatments, the Tukey's median comparison test ($p \leq 0.05$) was applied. We used the MINITAB 14 statistical software.

RESULTS AND DISCUSSION

Figures 1 and 2 show the climate variables —precipitation, temperature, and evaporation— registered during the year when the study was conducted.

During the dry season, only 11 mm of rainfall were recorded, while total evaporation (TEv) in February and March reached 119.1 and 127.3 mm, respectively, with maximum

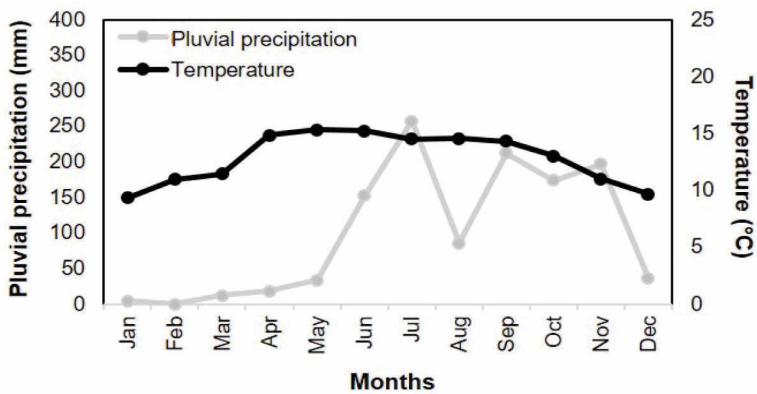

Figure 1. Pluvial precipitation and temperature in the municipality of Aculco, Estado de México, Mexico, during the development of the study.

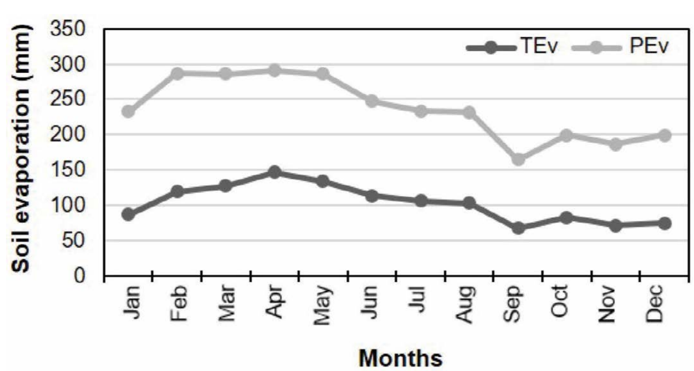

Figure 2. Soil total (TEv) and potential evaporation (PEv) in the production units.

average temperatures of 23.4 °C and 21.5 °C. Minimum average temperatures in February (with frost) and March were around 0 °C; during the rainy season (June and July), an average of 204.7 mm of rainfall was recorded, with approximate evaporation rates of 133 and 127 mm, maximum average temperatures of 22 °C, and minimum average temperatures over 8 °C.

The results for total evapotranspiration (TEv) and potential evaporation (PEv) obtained in this work are similar to those reported by Vacher *et al.* (1994), who assessed water balance in different plots during winter and found that TEv and PEv results were higher than in other seasons, which they related to the low temperatures recorded in this period. Soil evaporation is also related to the amount of vegetation cover, the soil surface moisturing before sowing, and the soil type (Castaño *et al.*, 2012; López-Báez *et al.*, 2018). During the rainy season, the vegetation cover does not eliminate evaporation —it can only reduce it. In fact, the thickness of this cover is a key factor in the drastic diminishing of evaporation (Kemper *et al.*, 1994).

Table 1 shows the results for the chemical and biological indicators found in the soil under different management practices and in different seasons.

pH

The pH of the studied soils is classified as moderate to strongly acidic (NOM-021-RECNAT-2000, 2002). The highest pH values were found in the control soils and no differences were recorded among seasons (dry, 5.91; rainy, 5.98). The lowest pH value was found in the maize plots (4.80). In 236 plots used to grow maize, López-Báez *et al.* (2019) reported pH values of 5.2 for 52% of soils, while the rest presented values lower than 5. The authors attributed these results to the soils' limited capacity to retain cations, resulting from their sandy texture and low organic matter content. In addition to their low capacity to store easily available K, Mg, and Ca, these cations are unprotected against the lixiviation process during the rainy season (Arcila-Pulgarín and Farfán-Valencia, 2010). Besides, soils can also be affected by agricultural practices (*e.g.*, the type of plow, the use of agrochemicals and of manure, and crop residue management), all of which is reflected on the acidification of plots in which maize is constantly grown.

Organic matter (OM)

The soil's OM presented significant differences ($p \leq 0.05$) between the different management practices and seasons. The soils cultivated with maize during the dry season had the highest concentration of organic matter; however, this value is classified as poor (NOM-21-RECNAT-2000, 2002), mainly because soils cultivated with maize are more prone to sediment and OM loss, as a consequence of their scarce vegetable cover at the beginning of their cultivation cycle (Fadul-Pacheco *et al.*, 2013). However, the amount of dry matter in the soil cultivated with maize increased during the rainy season, given the thicker vegetable cover on the soils. Meanwhile, the highest OM value was observed in the control soil during the rainy season. The results obtained in this work are similar to those reported by Wang *et al.* (2004), who mention that, after several years of annual crops, OM content diminishes because frequent farming affects OM inventories in the soil.

Table 1. Chemical and biological indicators of soils in the production units.

Soil parameter	**pH**			**Total nitrogen (g kg^{-1})**		
Soil system	**DS**	**RS**	**Mean**	**DS**	**RS**	**Mean**
Maize	4.80	5.30	5.05 b	0.8	1.9	1.35 c
Pasture	5.90	5.64	5.77 a	2.3	3.6	2.95 b
Control	5.91	5.78	5.84 a	2.6	3.1	2.85 a
Mean	5.53 b	5.57 a		1.9 a	2.86 b	
SEM Management	0.05*			0.01*		
SEM Season	0.04*			0.01*		
SEM Interaction	0.03*			0.02*		
Soil parameter	**Organic matter (g kg^{-1})**			**Total organic carbon (g kg^{-1})**		
Soil system	**DS**	**RS**	**Mean**	**DS**	**RS**	**Mean**
Maize	5.2	9.6	7.40 b	3.0	5.6	4.30 c
Pasture	7.8	2.9	5.35 c	4.5	7.5	6.00 b
Control	9.3	16.8	13.05 a	5.4	9.7	7.55 a
Mean	7.43 a	9.76 b		4.3 b	7.6 a	
SEM Management	0.02*			0.01*		
SEM Season	0.02*			0.01*		
SEM Interaction						

Soil parameter	**Carbon of microbial biomass in soil (mg C-CO_2 kg^{-1} dry soil)**		
Soil system	**DS**	**RS**	**Mean**
Maize	57.25	63.84	60.54 c
Pasture	120.29	134.38	127.33 b
Control	119.62	140.03	129.82 a
Mean	99.05 b	112.75 a	
SEM Management	1.35*		
SEM Season	1.11*		
SEM Interaction	1.91*		

*: Significant ($p \leq 0.05$). Different letters in each mean column and file indicate significant differences ($p \leq 0.05$). DS: dry season; RS: rainy season; SEM: standard error of the mean.

Total organic carbon (TOC)

TOC in the control soils during the rainy season is higher than in the dry season and in the case of the other two management practices. The amount of TOC in grassland soils is significantly different during the dry and rainy seasons. Magdoff and Weil (2004) report that grazing lands and grasslands improve soil TOC contents, which leads to an enhanced retention of organic-matter-related nutrients, better water relations, and an improved general functioning of soils.

The results found in this work for TOC in maize plots during the dry season are similar to those recorded by Salinas-García *et al.* (2002), who reported a direct relation between TOC and pluvial precipitation, with a lower TOC content during low precipitation periods. These results match the findings of Zinn *et al.* (2005), who found that soil TOC diminishes in intensive land-use systems with single-crop farming.

Microbial biomass carbon (MBC)

MBC in control soils during the dry season was no different from MBC in soils managed with grasslands, during the same season. Both managements showed an increase in MBC during the rainy season. Likewise, both had higher MBC values than soils used to grow maize. The results found in this work match those reported by Sparling *et al.* (1992), who found that MBC faced a 54-50 % reduction in the first 20 cm of soil cultivated with maize, as compared with soils used for the permanent cultivation of grassland. This is directly attributed to the quality of the OM added to the soil and to climate-related variations. Similarly, soil MBC is sensitive to changes brought about by tillage, crop rotation, and organic fertilizer usage, which have a positive effect on TOC and CSBM (Estrada-Herrera *et al.*, 2017). Low MBC values can be caused by a reduced C and N availability in the OM, resulting from an accelerated mineralization and the lixiviation of nutrients. These processes are favored by the destruction of soil aggregates due to frequent farming (Chaplot *et al.*, 2005).

Table 2 shows the results for the physical parameters of soil, which were significantly different ($p \leq 0.05$) between managements and seasons.

Bulk density (BD)

Control soils showed the lowest BD values (0.81 and 0.84 g/cm^3, respectively), which remained the same for both seasons. These data are similar to those recorded by Sánchez-Vera *et al.* (2003), who reported lower BD values in virgin forest soils than in soils with

Table 2. Physical indicators of the soils in the production units.

Soil parameter	Humidity (g kg^{-1})			Bulk density (g cm^{-3})		
Soil system	**DS**	**RS**	**Mean**	**DS**	**RS**	**Mean**
Maize	0.3	39.2	19.75 c	1.09	1.14	1.11 a
Pasture	0.4	52.7	26.55 b	0.96	1.07	1.01 b
Control	0.4	63.2	31.80 a	0.81	0.84	0.82 c
Mean	0.36 b	51.7 a		0.95 b	1.01 a	
SEM Management	0.10*			0.01*		
SEM Season	0.08*			0.01*		
SEM Interaction	0.14			0.02		

Soil parameter	Earthworm density (Number m^{-2})		
Soil system	**DS**	**RS**	**Mean**
Maize	5	11	8.0 c
Pasture	78	110	94.0 b
Control	7	16	11.5 a
Mean	30 b	45.66 a	
SEM Management	1.54*		
SEM Season	1.39*		
SEM Interaction	2.31		

*: Significant ($p \leq 0.05$). Different letters in each mean column and file indicate significant differences ($p \leq 0.05$). DS: dry season; RS: rainy season; SEM: standard error of the mean.

annual crops or grasslands, which they attributed to the fact that most soils with an undisturbed vegetation cover maintain an optimal BD.

These results show that farming has a high impact on changes in the distribution of primary particles and microaggregates in soils. Some authors have reported that, after seven or eight years of planting grassland, the physical properties of the soil are almost restored to their pre-modification levels (Sánchez-Vera *et al.*, 2003).

Earthworm density

Earthworm density showed higher values during the rainy season than the dry season; likewise, the values were higher in soils cultivated with grassland than in those cultivated with maize and control soils ($p \leq 0.05$). Domínguez *et al.* (2009) consider that an adequate earthworm density ranges from 100 to 500 individuals per m^2, and up to 2,000 individuals per m^2 in some temperate grasses. Earthworms are acknowledged as important indicators of soil health and environmental sustainability, since they play a key role in the improvement of soil fertility. A lower population of individuals per m^2 —which matches the data recorded in this work— was reported by Brito-Vega (2006), who mentions that farming the land tends to reduce earthworm population throughout time.

CONCLUSIONS

The alteration of soil quality by management practices influences the productivity and sustainability of the system through its impact on soil particle and microaggregate distribution, the depth of organic matter in the soil, microbial activity, earthworm density, and nutrient dynamics and availability. Meanwhile, crop rotation benefits soils, particularly when grasslands are sown after several years of being cultivated with maize.

Some of the parameters found in this work can be used as indicators of soil degradation and strengthen other parameters, in order to enhance the assessment of the sustainability of these systems.

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International positioning of Mexican mango, analysis of foreign trade competitiveness indices from 2005 to 2018

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ABSTRACT

Objective: To analyze the commercial competitiveness of Mexican mango in the global market, through the determination of growth rates of mango exports, imports and production, as well as the commercial competitiveness indices.

Design/Methodology/Approach: The production data were obtained from the Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera*, SIAP), and those of exports and imports from the International Trade Center of UNCTAD/OMC through TRADEMAP. The relative trade balance, the transability index, the export openness index, and the import penetration index were measured.

Results: The relative trade balance in Mexico was very close to 1. The transability index had an average of 0.2. The export openness index was positive every year. The import penetration index had values very close to 0. The exports increased 97% and the imports 30%.

Study Limitations/Implications: The available statistical records for the period of 2005 to 2018 were taken into account, considering all the varieties of mango produced and exported.

Findings/Conclusions: Mango is a primary product highly valued globally and, therefore, demand for this product has increased. Exports had a growing behavior, while imports are very low compared to the exports. The commercial balance was positive every year. Mexico evidenced an increase of competitiveness at the international level.

Keywords: Relative trade balance, transability, export openness, import penetration.

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INTRODUCTION

The global production of mango represented more than half of the total production of the main tropical fruits in 2018, with a production volume of 100.2 million tons (FAO, 2020). In 2016, Mexico was the fifth world producer with a volume of 1.88 million tons, and one of 25 mangoes consumed in the world was of Mexican origin (SAGARPA, 2017). India has been the main mango producer since 1960 and Mexico is the main exporter at


Image by Madhulika Rawal at Pixabay

the global level since 2008, and in 2018 it was the main supplier at the global level. In 2018, the main exporters were: Mexico with 385 thousand tons, Thailand with 250 thousand tons, Peru with 205 thousand tons, Brazil with 195 thousand tons, and India with 155 thousand tons (FAOSTAT, 2021).

In 2018, a decrease in the growth of exports was found globally, which is attributed to a contraction of 5% in exports from Mexico; in some producing zones of Mexico the adverse meteorological conditions did not only cause a reduction of the mango supply for exports, but caused a general estimated decrease of 2.7% in Mexican production (FAOSTAT, 2021).

In Mexico different varieties of mango are produced which have a high demand in the international market: Criollo, Diplomático, Manila, Haden, Kent, Keitt, Tommy Atkins and Ataulfo (CIATEJ, 2016). In 2016, Mexican exports represented a very significant percentage of mango imports in the United States (65.41%), Canada (63.86%), and Japan (47.66%) (SAGARPA, 2017). Therefore, the main markets for Mexican mango are the United States, Canada and, to a lesser degree the European Union and Japan (FAOSTAT, 2014).

Mexican mango does not have export duty for the United States, Canada, the European Union or Japan (SIAVI, 2017). Mango has a duty tax classification established by the World Customs Organization (OMC, 2021). In Mexico the duty tax classification has eight digits (SE/SIICEX, 2021), being tax fraction 08045003, which refers to all the varieties of mango that can be produced and exported from Mexico (SIICEX/CAAAREM, 2020).

In Mexico there is lack of knowledge about mango's commercial positioning, despite it being a key agricultural product; therefore, its commercial position should be determined through the analysis of competitiveness applying commercial indices. In this context, the objective of this study was to analyze the commercial competitiveness of Mexican mango in the global market, through the determination of growth rates of exports, imports and production, as well as through commercial competitiveness indices during the period of 2005 to 2018. The research hypothesis is that Mexican mango has increased its competitiveness in the international market, despite it not being the main world producer and the contraction in exports found in year 2018.

MATERIALS AND METHODS

The study was based on a quantitative analysis of the statistics of international trade, domestic production and international prices. The documentation of data was conducted through the following sources of information: the Agrifood and Fishing Information Service (*Servicio de Información Agroalimentaria y Pesquera*, SIAP, 2017; SIAP, 2021) with its Consultation Agrifood Information System (*Sistema de Información Agroalimentaria de Consulta*, SIACON, 2020), and the TRADEMAP tool developed by the International Trade Center (INTRACEN, 2006; ITC/TRADEMAP, 2021).

The statistical records of the period from 2005 to 2018 were taken into account, considering all the mango varieties produced and exported from Mexico. The research implied analyzing 14 observations, and because of this the inference generated through the analysis is significant to determine the behavior of commercial competitiveness.

The Inter-American Development Bank connects the competitiveness of an economy to the creation of the necessary conditions for entrepreneurial development and the sustainable increase of productivity and *per capita* income (BID, 2004). Likewise, the competitiveness of a nation depends on the ability of its industry to innovate and be improved (Porter, 2007).

The capacity of a nation to provide a good standard of living to its population depends on the ability of its enterprises to attain high levels of productivity; that is, it doesn't matter how much they have in resources, but rather how those resources are used (Porter, 1999). In turn, competitiveness resides in having a competitive advantage in relation to the other competitors (Porter, 2002).

An assumption is established that a nation is more competitive when, in addition to satisfying the domestic demand without the need to resort to imports, a high proportion of production is destined to exports. The methodology by Schwartz *et al.* (2007) was used for calculations of the indices that allow defining competitiveness.

The following variables were used for all the indicators: X, Export volume in tons; M, Import volume in tons; and P, Production in tons.

1. Relative trade balance (A). This indicator measures the relationship between a product's trade balance and its total trade for a country in the global market, or in a specific market. It consists in giving an idea of the condition of the chain in the market. It is assumed that an export chain is more competitive when it has to import fundamentally its prime material or intermediate goods.

$$A=(X-M)/(X+M) \tag{1}$$

2. Transability (T). If the value of T is close to -1, the recipients of the products can be important, since they are countries that are totally dependent on imports of the goods being evaluated. The countries with T close to 0 indicate a capacity close to self-supply. And the positive values indicate that they are exporters.

$$T=(X-M)/(P+M-X) \tag{2}$$

3. Export openness index (A. E.). This indicator serves to demonstrate to what degree countries are good exporters in function of their domestic consumption; that is, taking into account the national apparent consumption, it also measures the export vocation of the country and its capacity to build permanent comparative advantages. It is obtained using the following formula:

$$A.\,E.=X/(P+M-X) \tag{3}$$

4. Import penetration index (P. I.). It measures the relationship between the imports of a country with regards to its apparent consumption. The higher the index, it will

represent a higher purchasing capacity and therefore it signals that this country is less competitive. It is estimated with the following formula:

$$P.\ I. = M/(P+M-X) \tag{4}$$

To the extent that this indicator is higher, the competitiveness of the product is lower.

RESULTS AND DISCUSSION

Exports, imports, trade balance and production

Figure 1 shows the behavior of the commercial variables. The imports show that they are not significant compared to the exports and as consequence of this, the trade surplus is almost equal to the exports. On average, the imports compared to the exports only represent 0.8%.

The growth rate of exports was 97.1%. The imports had a growth of 30%. The net trade balance increased 97.72%. The main destinations of mango exports were the United States with 246.8 thousand tons, Spain with 70.6 thousand tons, Canada with 30.1 thousand tons, and Japan with 3.4 thousand tons.

Figure 2 shows mango production in Mexico from 2005 to 2018. In 2004 the production was 1,368,090.8 tons and in 2018 it was 1,867,297.7 tons, which represents a percentage increase of 36.48%. The main mango producing states in Mexico in 2018 were: Guerrero with 20.6% production, Nayarit with 17.2%, Chiapas with 14.95%, Sinaloa with 10.5%, and Oaxaca with 10.1%. The five prior states concentrate 73% of domestic production.

Relative trade balance index

Figure 3 shows that Mexico has maintained a growing competitive advantage; the calculated value of the relative trade balance approaches 1; the importance of exports in relation to imports is higher; and the country demonstrates it is a net exporter of the fruit. This indicator shows the exporting potential for mango from Mexico.

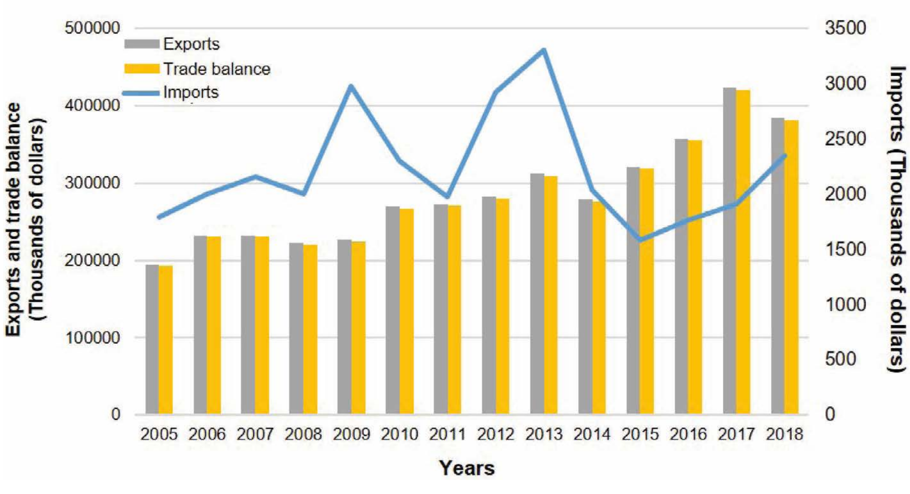

Figure 1. Behavior of mango exports, imports and trade balance in Mexico (thousands of dollars) in the period 2005 to 2018. Source: Prepared by the authors with TRADEMAP data (ITC/TRADEMAP, 2021).

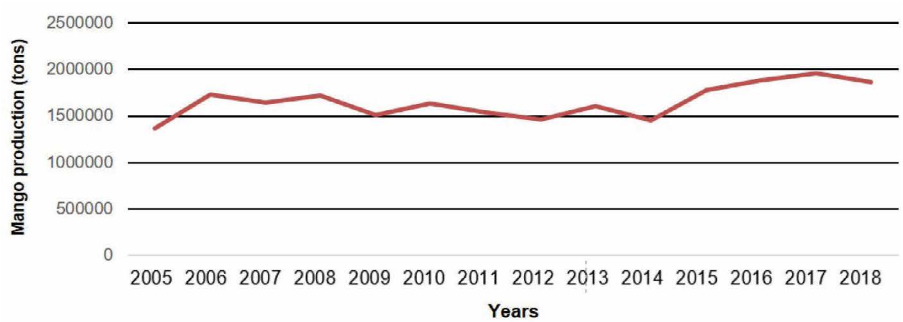

Figure 2. Mango production in Mexico in the period of 2005-2018. Source: Prepared by the authors with data from SIAP (2017; 2021).

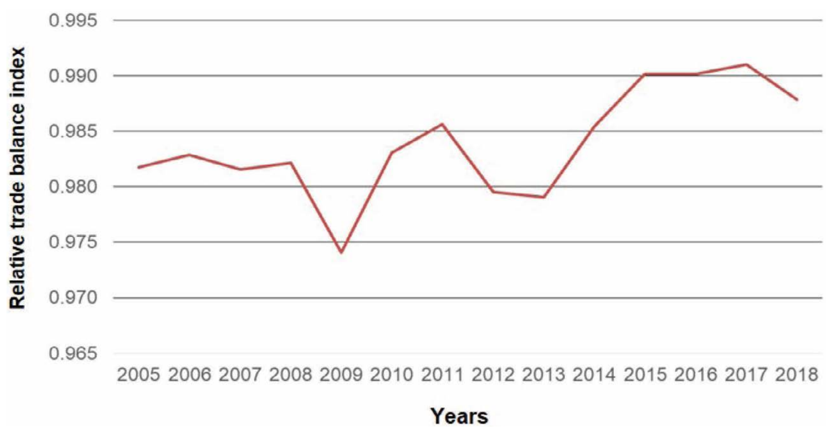

Figure 3. Relative trade balance index for mango from Mexico in 2005 to 2018. Source: Prepared by the authors with data from TRADEMAP (ITC/TRADEMAP, 2021) and SIAP (2017; 2021).

The year with the lowest relative trade balance index was 2009, with a value of 0.974 and the year that presented the highest index was 2017 with 0.991. In the study period, the average of relative trade balance index was 0.98.

Transability index

Figure 4 shows the evolution of transability which has advanced almost 10%; Mexico presents increasing competitiveness. The average transability index was 0.2, which reflects

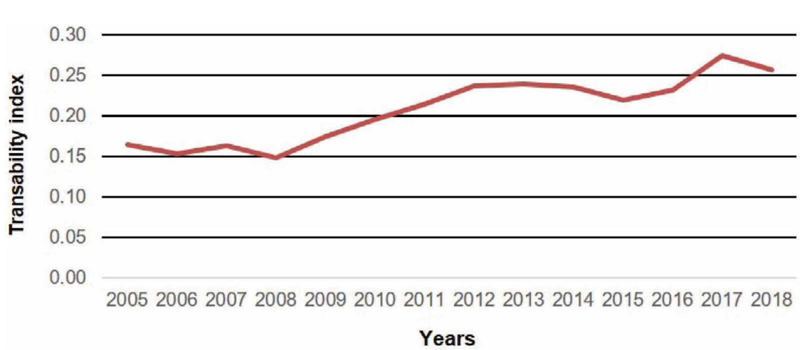

Figure 4. Mango transability index for Mexico in the period of 2005 to 2018. Source: Prepared by the authors with data from TRADEMAP (ITC/TRADEMAP, 2021) and SIAP (2017; 2021).

that mango production and trade is a competitive activity in the foreign market and shows an important growth and shows important growth. Likewise, it shows the local demand satisfied in general, despite the low imports, which shows the ability of mango producers to adapt to the new and more efficient forms of production.

Export openness index

The export openness index measures the participation of exports in the apparent consumption. Figure 5 shows that in each of the years analyzed this index is positive, which implies that the domestic demand in Mexico has been covered in each of the years.

The trend of the export openness index is positive and sustained. The findings show that Mexico has relative advantages in mango production for its export, and this is why it should be considered as a strategic product and its production and export should be promoted.

Import penetration index

Figure 6 shows that the import penetration index had values close to zero, which means that competitiveness of the productive sector of mango is higher; likewise, it indicates that the imports have a tendency to be zero meaning that they do not have an important position in the domestic consumption.

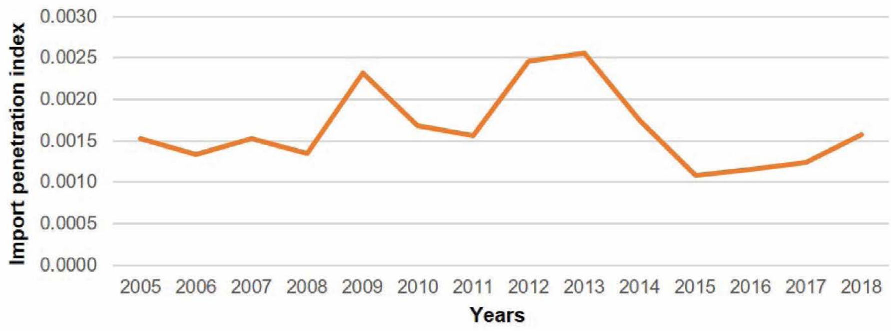

Figure 5. Export openness index of Mexican mango from 2005 to 2018. Source: Prepared by the authors with data from TRADEMAP (ITC/TRADEMAP, 2021) and SIAP (2017, 2021).

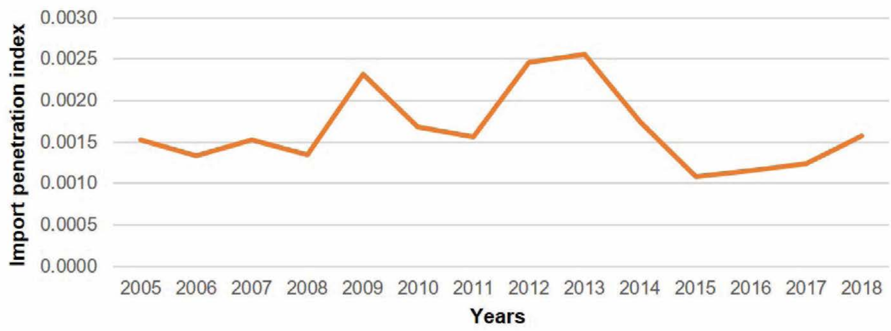

Figure 6. Import penetration index in the period 2005 to 2018. Source: Prepared by the authors with data from TRADEMAP (ITC/TRADEMAP, 2021) and SIAP (2017; 2021).

The mango product imports to satisfy the domestic consumption in Mexico are very low; the barely positive value of the index indicates that imports have existed throughout the analysis period, although such a small value evidences the low relevance of imports in the domestic market.

CONCLUSIONS

Mango has conserved its importance as an export product, and there is a wide range of production varieties, some of which originate in the country. Mexico remains as the number one exporter globally. Likewise, the relative trade balance index demonstrated that it is a competitive exporter with high exporting potential (exports increased), higher commercial positioning, and low importing potential. The transability index shows that the product is competitive and that the demand of the domestic market is widely satisfied, which is why there is no need to resort to imports. Export openness indicates that there is exporting vocation, and that there are permanent advantages in production and export due to the improvement in mango's production chain.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of tropical cattle production units: Effect of sanitary management

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ABSTRACT

Objective: To characterize dual-purpose cattle production units (PUs) and to identify the sanitary management that producers use with their herds.

Design/Methodology/Approach: Sixty (60) cattle producers were interviewed; the questions were related to the characteristics of the PUs and the sanitary status. Four serum samples from the largest herd were sent to the laboratory to detect diseases.

Results: All the cattle producers surveyed were men between 20 and 50 years old. Of them, 40% have been devoted to the activity for 11 to 25 years. The PUs have on average 25 animals, 45% of producers have between 21 and 30 cows, and the highest percentage of animals are breeds Gyr (35%) and Sardo Negro (30%). Of producers, 65% tend to their sick animals. On the other hand, the laboratory studies showed that three cows were positive for Bovine Respiratory Syncytial Virus (BRSV), two cows were positive for type 1 bovine herpes virus (BoHV-1), or Infectious Bovine Rhinotracheitis (IBR) and parainfluenza 3 (PI3), and finally all the cows were positive for *Leptospira* sp. Hardjo.

Study Limitations/Implications: Under the conditions in which the study was conducted, it is costly to send samples to the laboratory. The producer with the largest herd financed the laboratory tests of the cows sampled. This information is useful for local producers with regards to the characteristics in which they produce them.

Findings/Conclusions: Dual-purpose cattle producers in the tropics produce at a small scale; and one of the largest PUs presents problems of abortive diseases.

Keywords: animal production, small scale, socioeconomic study, health, microorganisms.

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INTRODUCTION

In recent years, concern over food security has increased in Mexico and the world, due to the increase in population (9 billion inhabitants for the year 2050); therefore, there will be higher demand for beef (FAO, 2009). Presently, Mexico has 31 million heads of cattle: dairy (4.6%), beef and dual purpose (58.6%) (SIAP, 2016). The state of Guerrero has 1 million 3 thousand heads, in 42,000 production units (PUs), distributed regionally in Tierra Caliente (39%), Norte (18%), Costa Chica (16%), Costa Grande (16%) and the rest of the state (11%) (FIRA, 2017). In the country, to breed cattle there are different production systems, classified according to the technology used, from modernized to backyard systems (Rubio-Lozano *et al.*, 2013). Based on productive affinity, production systems for beef, milk and dual purpose were observed, where the main objective is producing milk and beef from the sale of calves and waste cows (SIAP, 2014). In cattle production systems, crosses between the species *Bos indicus* (Swiss American, Charolais and Simmental) and *Bos taurus* (Gyr, Guzerat, Brahman and Sardo Negro) are used, with the European breeds standing out as paternal lines and the Zebu as maternal (Castellanos-Gómez *et al.*, 2016). Breeding is conducted to take advantage of the rusticity and adaptation of livestock to tropical environmental conditions (Román-Ponce *et al.*, 2013). It is important but not sufficient to characterize and consider the size of cattle PUs, but rather also the amount of economic resources that are invested; it is also important to consider management, development of the activity, use of resources, and differences imposed by the producers' culture (Vilaboa and Díaz, 2009; Ruiz-Ortega *et al.*, 2021). Likewise, it is necessary to identify the socioeconomic characteristics of producers, size of the PU, and the commercialization of beef, milk and milk byproducts (Orantes-Zebadúa *et al.*, 2014; Ruiz-Ortega *et al.*, 2021). Some studies about the characterization of milk and dual-purpose cattle PUs consider them small-scale and sustainable production systems fit for tropical conditions (Orantes-Zebadúa *et al.*, 2014; Severino-Lendechy *et al.*, 2021). On the other hand, it is important to consider the sanitary state of cattle PUs, since cattle herds being healthy and producers not losing economic resources when covering treatment for the diseases depend on this. Indeed, in the tropics the diseases that affect dual-purpose cattle are brucellosis, tuberculosis, para-tuberculosis, bovine viral diarrhea (BVD), papillomatosis, leptospirosis (*Leptospira* sp.), among others (Gutiérrez-Hernández *et al.*, 2020; Ponce-Covarrubias *et al.*, 2021). Taking into consideration these diseases of economic importance for producers of the Mexican tropics, there is strong evidence of the presence of reproductive diseases, characterized by causing infertility, embryo death, malformations and abortions, provoking serious economic losses for producers (McGowan and Kirkland, 1995; Mellado *et al.*, 2021). These are cattle diseases with multiple etiologies (viral and bacterial), such as the bovine viral diarrhea (BVD) and bovine 1 herpes (BHV-1) viruses, the causal agent of IBR, *Brucella* sp. and *Leptospira* sp. bacteria, which in addition to generating great losses for producers tend to be zoonotic diseases (Wounda *et al.*, 1999; Khodakaram-Tafti and Farhanikish, 2017). Because of this, the objective of this study was to characterize dual-purpose cattle production units (PUs), and to identify the sanitary management that producers use in their herds.

MATERIALS AND METHODS

Study area

This study was carried out in the ejido Coacoyulichán "Rancho el Aguacatillo", municipality of Cuautepec, Guerrero, Mexico. The place belongs to the tropics and is located between geographic coordinates 16° 51"48"' LN and 99° 53' 24" LW. The highest average temperature takes place during the summer months (39.5 °C) and the lowest during the winter months (16 °C) (García, 2004).

Description of the study herd

The dual-purpose cattle producer has been working for 27 years and owns a cross of Swiss American and Sardo Negro. Currently, there are 185 cattle, of which 100 are multiparous cows, 30 replacement heifers, 50 calves for sale, and 5 registration bulls. The animals are vaccinated against the diseases of paralytic rabies (BIO ZOO, Zapopan, Jalisco, Mexico) and blackleg each year (Triple bovine bacterial, Zapopan, Jalisco, Mexico). Every 6 months parasites are removed (Sanfer® Ivermectin Animal Health, 1 mL for every 50 kg, Tlacopac, Mexico City, Mexico) and supplied with vitamins (Super B Complex, Tornel Laboratories, Naucalpan, Estado de México, Mexico). The reproduction is carried out by natural mounting and the animals do not receive complementary feeding in any season of the year. When it comes to productive aspects, there are normally 30 dairy cows from which milk and cheese can be sold, while calves and waste cows are for local sale. In the herd, there is no technical advice from a veterinarian.

Design and application of the survey

During the months of February to May, 2019, 60 dual-purpose cattle producers were interviewed. For that purpose, the formula proposed by Otzen and Manterola (2017) was applied, where the sample was limited by using a simple random sample and each Production Unit (PUs) was considered as an experimental unit represented by each livestock producer. The questionnaire was constructed with 21 items and divided into five sections: data of the owner, data of the PUs, description of the current production, characteristics of the PUs, and animal health (Ruiz-Ortega *et al.*, 2021). The study variables were classified into: socioeconomic, sanitary and commercial, the types of variables used in this study were categorical and numerical (Agresti, 2013). It was decided to work with the PUs described in the section "Description of the study herd" since it presented a larger number of animals, more sanitary problems and deaths in its livestock.

Anamnesis or clinical history

According to the survey applied, related to the health of cattle PUs, most of the producers mentioned not knowing about the diseases that affect their livestock (respiratory or digestive problems). Because of the situation experienced in the herd described in the section "Description of the study herd", the interview showed that each year they had a high number of deaths of cattle of different ages; however, 28 cattle had died in the last two years. Therefore, anamnesis and inspection of the animals was conducted with the owner of this livestock, performing a physical exploration of the cattle affected, females that had previously suffered abortions, nervous problems, respiratory problems, diarrheas, and animals that recently presented some disease with evident clinical signs. Because of the information obtained, the veterinarian decided to take some samples and send them to the histopathology laboratory (Indexx Laboratorios, Puebla, Mexico).

Taking and sending samples to the laboratory

Blood samples from the coccygeal vein were taken with Vacutainer tubes from four multiparous cows with background of abortions, diarrhea and respiratory problems. The tubes with the serum were marked with water-resistant ink, wrapped to fix the tape and placed in an ice box. The same day the samples were sent to the laboratory where a serologic diagnosis of the diseases was requested: bovine syncytial respiratory virus (BSRV), infectious bovine rhinotracheitis (IBR), parainfluenza 3 (PI3), leptospirosis and brucellosis, to the laboratory of Biología de Investigación Aplicada S. A. de C. V. (Indexx Laboratorios).

Statistical analysis

The information about the characterization of dual-purpose cattle PUs was analyzed using descriptive statistics; the percentage participation of each variable in the population was determined through frequency tables. The database was processed through multivariate analysis (conglomerate) for its grouping and classification. All the analyses were conducted with the SAS version 9.0 software (SAS Institute Inc., Cary, NC, USA). The results are presented in percentages, in the case of diseases, as positive or negative.

RESULTS AND DISCUSSION

Characterization of dual-purpose cattle PUs

In this study it was found that cattle producers in ejido of Coacoyulichán are between 20 and 50 years old, of which 100% are men. Similar results were found in dual-purpose cattle PUs in the states of Morelos, Sinaloa and Veracruz (Cuevas *et al.*, 2012; Chalate-Molina *et al.*, 2020). Ruiz-Ortega *et al.* (2021) found similar results to those in this study and to the ones mentioned before, in dairy cattle PUs in Valle de Tulancingo, Hidalgo. Contrary to these results, Granados-Rivera *et al.* (2018) mentioned that in the state of Tabasco the producers are older than 54.5 years and all men. Talking about the economically active population sector, the producers are of an adequate age (35 years), since for producers who are older than 50 years it is difficult to adopt new technologies to improve their cattle PUs.

Of the producers surveyed, 10% do not have any studies, 65% have primary school studies, and 25% have secondary school studies; 35% have been devoted to this activity for 5 to 10 years and 40% for 11 to 25 years. Finally, 100% of the producers have lands of their own for grazing (Table 1). When it comes to the producers' schooling, the results were similar to those found in the state of Tabasco, where most of the producers have primary school studies (52%). In the states of Hidalgo and Veracruz, 35.7 and 22% of the producers have higher studies. In this study, the producers have been devoted to this activity between 11 and 25 years, and all the producers have ejido lands for grazing. Consistently with these results, Granados-Rivera *et al.* (2018) indicate that most of the lands (74%) where they graze their livestock are within an ejido. For their part, Ruiz-Ortega *et al.* (2021) mention that lands are privately owned (57.1%). Regarding the time they have been devoted to the activity, Méndez-Cortés (2019) indicate that it was 23 years. Therefore, the experience, the form of management, and the cultural patterns influence the destination of these dual-purpose cattle PUs in the tropics.

On the other hand, it was also evidenced that the PUs have between 10 and 40 animals. Of the producers, 16% have less than 10 animals, 22% have between 11 and 20 animals, and 45% have between 21 and 40 cows (Table 2). For their part, the highest percentage of animals is of breeds Gyr (35%) and Sardo Negro (30%), with milking of between 21 and 30 cows (75%). Similar information was reported by Granados-Rivera *et al.* (2018) where they explain that on average they have 39.5±24.7 animals. In turn, Ruiz-Ortega *et al.* (2021) mention that in the Tulancingo Valley, Hidalgo, producers have on average 20 animals. On the other hand, in the state of Hidalgo there are breeds directed at milk production, such as Holstein, Brown Swiss and Jersey (Ruiz-Ortega *et al.*, 2021). For its part, in the tropics as in the states of Veracruz and Chiapas, they are Zebu breeds (Gyr, Sardo Negro, Guzerat, among others) with crosses with *Bos taurus* to take advantage of the hybrid vigor and rusticity of the animals (Román-Ponce *et al.*, 2013; Orantes-Zebadúa *et al.*, 2014).

Finally, the reproductive management of most of the PUs is done with natural mounting (88%) and when it comes to diseases, the livestock producers tend to their sick animals themselves (65%) (Table 2). Regarding the nutritional and sanitary management, most of the producers do not supplement their livestock. In this sense, Orantes-Zebadúa *et al.* (2014) mention that they supplement the animals with nutritional deficiencies during the time of drought. For their part, Ruiz-Ortega *et al.* (2021) express that they use reproductive technologies, dietary supplementation, and require advice from a veterinarian to treat the diseases. This situation is probably due to the age or schooling of the producers, since in the state of Guerrero most of the producers do not have any

Table 1. Socioeconomic characteristics of cattle producers.

Variable	Class	%
Age	Between 20 and 30 years	40
	Between 31 and 40 years	40
	Between 41 and 50 years	10
	More than 50 years	10
Sex	Male	100
Educational level	No studies	10
	Primary	65
	Secondary	25
Years dedicated to the PUs	Between 5 and 10 years	35
	Between 11 and 25 years	40
	More than 25 years	25
Grazing paddocks	Own	100
	Rented	0

Table 2. Characteristics of the dual-purpose cattle PUs.

Variable	Class	%
Number of animals	Less than 10	16
	Between 11 and 20	22
	Between 21 and 40	45
	More than 41	17
Breeds of cows	Swiss American	25
	Gyr	35
	Sardo Negro	30
	Others	10
Milking cows	10 a 20 cows	28
	21 a 30 cows	55
	More than 30 cows	17
Reproductive management	Natural copulation	88
	Reproductive biotechnologies	12
Disease management	Consultation with the ZVD	10
	Consult another farmer	25
	Attend personally	65
What vaccinations do you give your cattle?	Bacterin Biobac® 7 ways	100
	Others	0

studies or primary school studies. For their part, producers from the state of Hidalgo and Veracruz have higher studies and accept new technologies to improve their cattle PUs.

Producers of tropical cattle generally do not accept advice from veterinarians. Therefore, a high percentage of the livestock die from diseases of unknown etiology and the producers have important losses (Escamilla *et al.*, 2007). The most common diseases that affect tropical cattle are blackleg, pneumonia, diarrhea, mastitis, anaplasmosis, parasitosis, derriengue and papillomatosis, among others (Orantes-Zebadúa *et al.*, 2014; Ponce-Covarrubias *et al.*, 2021). This study emphasized asking producers about who treats their sick animals; however, some producers mentioned diseases that they offered treatment for: respiratory problems, diarrhea, nervous problems, parasites and sporadic abortions (Table 3). Most of the producers treat their animals or take advice from another producer to deal with the diseases. However, despite applying vaccines, they have high mortalities in animals of different ages annually, situation that affects the producers' economy. These losses keep them from investing in the PUs, particularly in the sanitary aspect since commonly producers do not know the etiological agent of the diseases that affect their livestock. Therefore, it is important for them to have a deworming and vaccination calendar, to avoid the loss of the cold chain for the vaccines in order to prevent their deactivation and thus to reduce the mortality.

Disease diagnosis

In the country, the etiologic agent in more than 70% of the abortions of dual-purpose cattle is unknown, in addition to health problems that compromise the productivity of

Table 3. Effects from cattle deaths for the producer.

Variable	Class	%
Number of deaths per year	Less than 10	36
	Between 11 and 20	42
	Over 21	22
Time of the year	Rains	40
	Dry	60
What clinical signs does the cattle before dying?	None	5
	Abortions	51
	Nerve problems	15
	Respiratory problems	22
	Others	7
Have vaccinated animals died?	Yes	35
	No	65
How does the death of your animals affect you?	Economic	37
	Replacements	32
	Self-esteem	19
	Others	12

livestock herds (Escamilla *et al.*, 2007). Reproductive diseases are the ones that have the greatest effect on cattle PUs, since they place at risk the availability of calves and increase the production cost for producers (Rojo-Rubio *et al.*, 2009). In this study, the laboratory results confirmed three positive cows for the bovine syncytial respiratory virus (Table 4). Also, two cows were positive and two negative for antibodies against the glycoprotein B (gB) of type 1 bovine herpes virus (BoHV-1), causal agent of bovine infectious rhinotracheitis and IgG+IgM Parainfluenza 3(PI3). These diseases belong to the bovine respiratory complex with multifactorial etiology. Some studies mention that the etiological agents associated with reproductive disorders in cows include viral agents [bovine viral diarrhea (BVD), infectious bovine rhinotracheitis (IBR)] and bacterial agents [*Brucella abortus* and *Leptospira* sp. (Hardjo, Pomona, among others)], which affect the bovine females with high percentages of abortions (Martins and Lilenbaum, 2017; Larghi, 2018; Valas *et al.*, 2019). On the other hand, *Leptospira* sp. Hardjo 1:100 strain was identified for the four cows and the Pomona 1:100 strain for one positive cow (ID 001204), finding another two strains to which the same cow (ID 001204) was exposed (Grippotyphosa and Tarassovi 1:200) (Table 4). It is common that in cattle, the leptospirosis found frequently is the serotype Hardjo, because bovines are reservoirs of this serotype and contagion takes place between them from direct contact (Carmona-Gasga *et al.*, 2011). Some studies, where the serum prevalence of leptospirosis in tropical regions was determined, found high percentages for the serotype Hardjio (54.4%) (Escamilla *et al.*, 2007; Carmona-Gasga *et al.*, 2011). There are factors that favor the survival of *Leptospira* outside of the hosts, such as environmental moisture, causing for animals to acquire the infection. In the PUs sampled the number of sick animals from leptospirosis is unknown, since only four cows were sampled. However,

Table 4. Abortive diseases in the production unit (PU) of the study ranch, 'El Aguacatillo.

ID	Disease	Interpretation	Results
	S/P%[1]		
001204 400125 140126 181322	Bovine Respiratory Syncytial Virus	24.64 55.08 −1.60 66.30	Positive Positive Negative Positive
	Optical Density[2]		
001204 400125 140126 181322	Bovine herpesvirus type 1 (BoHV-1)	0.954 0.042 0.969 0.040	Negative Positive Negative Positive
	S/P%[3]		
001204 400125 140126 181322	Parainfluenza -3(PI3)	13.82 58.95 11.10 83.55	Negative Positive Negative Positive
	Titles of 1:100 (+)[4]		
001204 400125 140126 181322	Leptospira *SP* Bovine	Hardjo Hardjo Hardjo Hardjo	Positive Positive Positive Positive

[1] Interpretation of the Bovine Syncytial Respiratory Virus (BSRV) Kit, S/P% value lower than 20 is negative; S/P% value higher than or equal to 20 is positive.
[2] Optical density (O.D.) higher than 0.5 is positive, and lower than or equal to 0.5 is negative.
[3] $S/P<20$ value is negative, $S/P\geq 20$ value, and <40 is positive 1, $S/P\geq 40$ value and <60 and 80 is positive 3, $S/P\geq 80$ value and <100 is positive 4 and $S/P\geq 100$ value is positive 5.
[4] Titration of 1:100 is considered positive (presence of antibodies). In the four cows the Hardjo strain was found, and in cow one (ID 0011204) the Pomona (1:100), Grippotyphosa and Tarassvi strains were also found with titrates of 1:200.
Reference: Myers (1985).

it is important to make evident the presence of the disease in the cattle herd. In general, the results from this study show the presence of BVD, IBR and leptospirosis, which can be due to the purchase of wombs or bulls from infected herds, and because quarantines or immunization of animals are not carried out.

CONCLUSIONS

The cattle producers from ejido Coacoyulichán, municipality of Cuautepec, Guerrero, produce at small scale with low technology, without training to address productive and sanitary problems of the cattle PUs. Likewise, the cattle PUs show that there are problems in the diagnosis and knowledge of diseases, as well as in the application of treatments of sick animals. Finally, important abortive diseases for herd health and the producer's economy were found in the PUs where laboratory tests were made. It is necessary to train the producers for them to make correct decisions at the productive and sanitary level, contributing to suggest vaccination calendars, taking care of the cold chain for vaccines so they do not deactivate. It is also necessary to advise producers who are owners of animals with abortive diseases, so that they do not spread to surrounding PUs, as well as to sample and corroborate suspicious cases in the veterinary histopathology laboratory.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Broadleaf weeds associated with the cultivation of habanero chili (*Capsicum chinensis*) in the Yucatan Peninsula, Mexico

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ABSTRACT

Objective: To identify the weeds associated with habanero chili cultivation in the Yucatan Peninsula that can be considered pest hosts.

Design/Methodology/Approach: Composite soil samples were obtained from plots established with habanero chili in the states of Campeche, Yucatán, and Quintana Roo. The samples were taken to a greenhouse, where the weeds emerged and developed. The weeds were identified through images and with the support of herbariums. Indeces were used to identify the state with the greatest floristic diversity. DNA from symptomatic plants was obtained to confirm the presence of begomovirus.

Results: The Asteraceae family stood out among the 31 weed families that were identified. The floristic composition was different in the three states. The dominant species were *Amaranthus spinosus*, *Parthenium hysterophorus*, and *Acmella oppositifolia* in Campeche, Yucatán, and Quintana Roo, respectively. The state with the greatest diversity and richness was Yucatán. Twenty-six out of the thirty-one symptomatic samples tested positive for begomovirus.

Limitations/Implications: The seed banks have constant variations from one cycle to another; consequently, it is not possible to obtain the total of the species present in the samples.

Conclusions: It is necessary to establish the weed species present to propose improvements in technological packages and achieve sustainable management.

Keywords: seed bank, sustainable management, floristic diversity.

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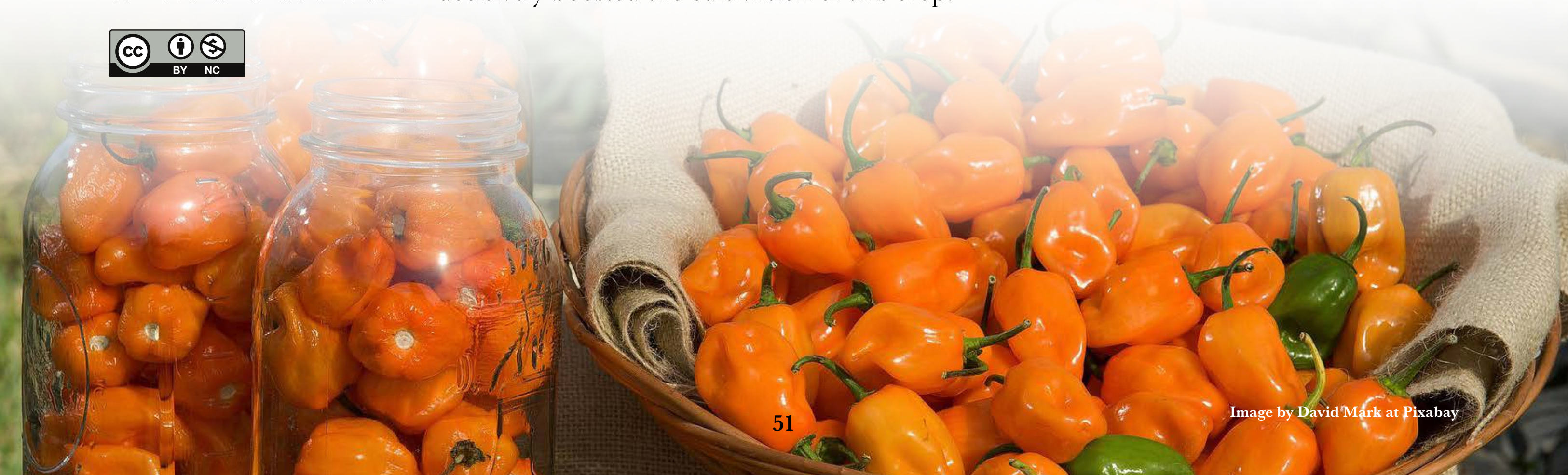
INTRODUCTION

The habanero chili cultivation is economically important in the Yucatan Peninsula. In 2020, the national production surpassed 21,973.81 tons, 22% of which were produced there (SIAP, 2021). In 2010, the Yucatan Peninsula was awarded the habanero chili denomination of origin in the Diario Oficial de la Federación (DOF 06/04/2010), which decisively boosted the cultivation of this crop.



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In the study region, this crop is still grown in the open field; this situation implies some disadvantages in terms of the presence of pests and diseases, although some producers are changing to protected agriculture conditions. A frequent problem is the presence of weeds —which become reservoirs of pests and diseases, including the whitefly (*Bemisia tabaci* Genadius) species complex and begomoviruses (Jiang *et al.*, 2004)— and the competition for nutrients, water, and light which reduces the productive potential of the crops (Zita-Padilla, 2008). In some studies about weeds, up to 50% productivity losses have been reported (Cotero, 1997). Other authors report total losses as a result of the competition between weeds and such crops as cotton, peas, corn, and carrots (Zamorano *et al.*, 2008; Blanco *et al.*, 2014; Cardenal-Rubio *et al.*, 2016). Weeds can cause direct and indirect damages (Liebman *et al.*, 2001). The weeds present in the crops can host disease-transmitting insects and viruses that can infect the habanero chili (Ying and Davis, 2000; Capinera, 2005; Dimeska *et al.*, 2011; Orfanidou *et al.*, 2016).

Studying seed banks is a tool to carry out an efficient control of pests and avoid the crop-weeds competition (Vargas-Gutiérrez and Blanco-Metzler, 2012; Zepeda-Gómez *et al.*, 2015; Caproni *et al.*, 2015; Cardenal-Rubio *et al.*, 2016). Between 70,000 and 90,000 seeds per square meter can be found within the first 15 cm of agricultural soil; this can provide a perspective on the type of weed and the potential control. The time and number of seeds kept in the soil is variable (Shiferaw *et al.*, 2018). The number of weeds present is very similar between the open field and the evaluation of the seed bank carried out in the greenhouse (Ribeiro-Mesquita *et al.*, 2016). Consequently, the objective of this work was to identify the seed bank weeds present in habanero chili fields in the Yucatan Peninsula.

MATERIALS AND METHODS

In order to determine the diversity of weeds present in habanero chili crops, two soil samples were obtained: the first, at the beginning of the 2013 wet season (June and July); the second, during the 2014 dry season (February and March). Both collections were made before the sowing started. Of the five sampling sites, three are located in the municipalities of Othón P. Blanco (Quintana Roo), Tizimín (Yucatán), and Muna (Yucatán) and have a warm subhumid climate with summer rains, with an intermediate humidity regime, and a >10% winter rainfall with regard to the annual total [Aw1(x')]. The other two sites —located in the municipality of Cayal in Campeche (Aw1) and in Mocochá in Yucatán (Aw0)— are less humid and have a warm sub-humid climate with summer rains and winter rains ranging from 5 to 10% of the annual total (García, 1997).

The soil type found in Tizimín and Mocochá is Rendzinas with medium texture, while in Chetumal the soil has a fine texture. In Uxmal the soil is eutric Nitisol with a fine texture; while Cayal has orthic Solonchak type soils with a fine texture (INIFAP-CONABIO, 1995).

The samples were taken in the established 400-m^2 plots, along four X-shaped diagonal transects, starting from the center of the plot and moving towards the edge. Each sample was taken 5 m apart from the previous one, at a 20-cm deep soil extraction point. Each of the nine samples per plot had a 20×20×20 cm volume.

In order to determine the diversity associated with the plots, the soil samples were placed in 53×26×6 cm trays with 1 centimeter of inert substrate and Agrolita™ substrate, under confined conditions in a greenhouse. The seeds were subject to constant irrigation in order to promote the germination and phenological development of the plants; they were transplanted into 250-g bags until the reproductive stage, when they were identified. Images of the adult plant with flower, fruit, and seed were obtained. The photographs of the complete plants were taken with a semi-professional camera (Canon model SX50HS) and a professional camera (Sony SLT-A57). The images of the seeds were obtained with a stereoscope (Carl Zeiss) with an adapted camera (Axiocam ERC).

The images and the *in vivo* plants were used to identify the weeds, consulting the online herbarium databases of national and international institutions such as the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO, http://www.conabio.gob.mx/malezasdemexico/2inicio/paginas/lista-plantas.htm), the Centro de Investigación Científica de Yucatán (CICY, http://www.cicy.mx/sitios/flora%20digital/index.php); the Missouri Botanical Garden (http://www.missouribotanicalgarden.org/gardens-gardening/our-garden/plant-records.aspx), the Instituto de Ecología, A. C. (INECOL, http://www.inecol.edu.mx/inecol/index.php/es/ct-menu-item-1/ct-menu-item-5/herbario), and the Herbario Nacional de México of the Universidad Nacional Autónoma de México (MEXU, http://www.ib.unam.mx/botanica/herbario/).

The number of total specimens per year at each sampling point was obtained, as well as the number of families, genera, and species. Biological indices were calculated to measure the alpha diversity defined by Whitakker (1972), considering that the environments are homogeneous among themselves. The species richness (Formula 1) and Margalef (2) indices were calculated, as well as the Simpson (3) and Shannon-Wiener (4) proportional abundance indices (Moreno, 2001).

Formula (1): Total number of species from the census of the sample by entity.

Formula (2): $D_{Mg} = \frac{S-1}{\ln N}$

Where D_{Mg} is the Margalef index, S is the number of species, and N is the total number of individuals.

Formula (3): $\lambda = \sum p_i^2$

Where λ is the Simpson index and p_i is the proportional abundance of i species, *i.e.*, the number of individuals of the i species divided by the total number of individuals in the sample.

Formula (4): $H' = -\sum p_i \ln p_i$

Where H' is the average degree of uncertainty when forecasting to which species an individual randomly selected from the collection will belong (Shannon-Wiener index): *i.e.*, the abundance, taking into account the importance of each species, with respect to the total number of species in the community.

Symptomatic plants were collected at the sampling sites. DNA was isolated with the modified CTAB protocol (Dellaporta, 1983); while the degenerate AV494/AC1048 primers that flank the viral coat protein were used to determine the presence of begomovirus (Holguín-Peña *et al.*, 2004).

RESULTS AND DISCUSSION

During the two years, 5,583 specimens were obtained from the soil samples; the specimens were classified into 31 families with 69 genera and 91 species. The predominant family was Asteraceae with a total of 16 genera and 1,682 identified specimens. Of the Fabaceae family, 7 genera were identified and 70 specimens were recorded. Nearly 1,200 specimens of the Amaranthaceae family were identified (Figure 1).

In the state of Campeche, the most important family was Amaranthaceae, with 50% of the total identified specimens: two species, *Amaranthus spinosus* L. and *Amaranthus polygonoides* L., stood out with 350 and 186 specimens, respectively. This family has proved to be highly adaptable to the Yucatan Peninsula: 12 *Amaranthus* species have been identified as invasive weeds in the agricultural land of the region (Sánchez-del Pino *et al.*, 2019). Meanwhile, the Asteraceae family was predominant in Yucatan (35% of specimens), followed by the Euphorbiaceae family (20%). The most abundant species of each family were *Parthenium hysterophorus* L. (731 specimens) and *Euphorbia dioica* L. (373 individuals). Finally, the Asteraceace family was predominant in Quintana Roo, with 46% of the specimens: *Acmella oppositifolia* L. (290 specimens) was the most abundant species.

Several authors report that the Asteraceae family is predominant in both annual and perennial agricultural crops (Vargas-Gutiérrez and Blanco-Metzler, 2012; Castillo *et al.*

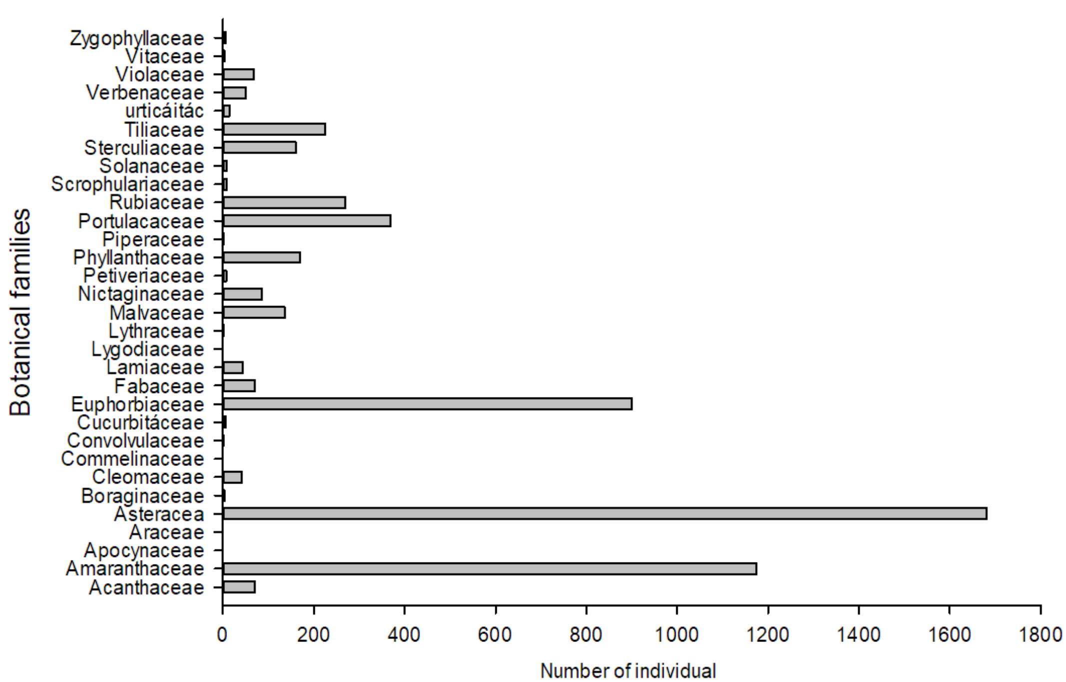

Figure 1. Number of specimens identified per family in the soil samples obtained from habanero chili plots in the Yucatan Peninsula.

2015; Castro-Cepero *et al.*, 2019). Establishing the type of floristic variability within the crop is of the utmost importance, since it will allow an efficient pest control management, as reported by Castillo *et al.* (2015) who identified the entomofauna of the weeds present in various crops, classifying more than 50% as crop pest insects and only 3.79% as parasitoid insects.

The distribution of weeds was heterogeneous in each state: of the total specimens obtained, 3,595 were found in Yucatán, 1,292 in Campeche, and only 696 in Quintana Roo (Figure 2). The previous order was maintained in the values of the biological indices calculated for richness and abundance (Table 1).

Yucatan has a greater richness and abundance than Quintana Roo and Campeche, owing to the higher number of species and the high possibilities of finding them. Even though the soil in the three sampled sites can maintain an active seed bank, the environmental conditions would explain the differences between them. Consequently, although the conditions in Mocochá, Yucatán, are drier (Aw0), the secondary vegetation of the surrounding lowland rain forests compensates for this by being a constant source of diasporas. In contrast, Quintana Roo, despite its humid climate [Aw1(x')], has a physical

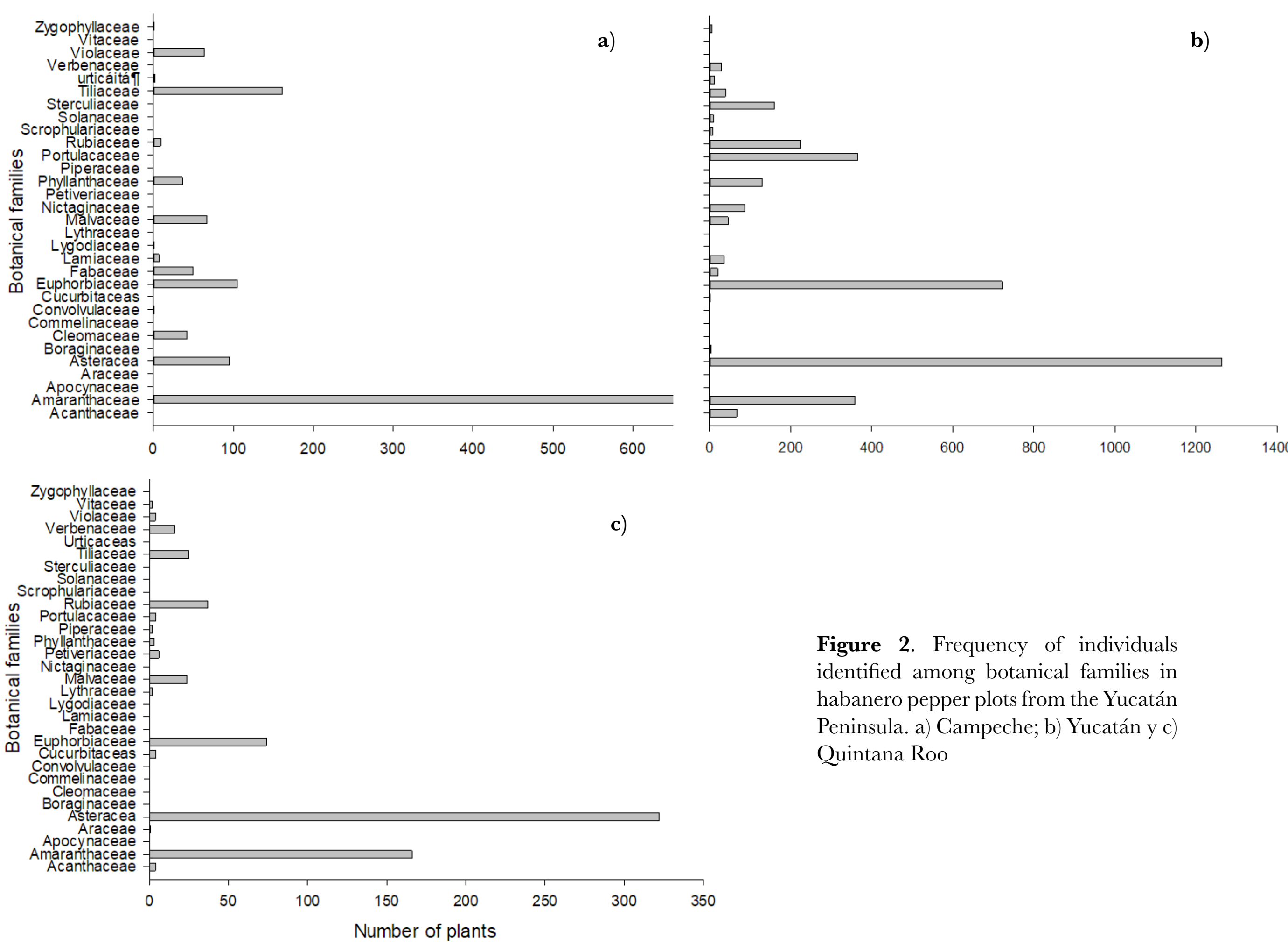

Figure 2. Frequency of individuals identified among botanical families in habanero pepper plots from the Yucatán Peninsula. a) Campeche; b) Yucatán y c) Quintana Roo

Table 1. Alpha diversity biological indicators for each federal entity.

County	Richness		Abundancia	
	R	Dmg	λλ	H'
Campeche	38	5.1648	0.8838	2.6582
Yucatán	73	8.7831	0.9176	2.9828
Quintana Roo	42	6.2544	0.7707	2.1826

R=Specific richness, DMg=Margalef index, λ=Simpson index, H'=Shannon index.

barrier, caused by the planting of *Cedrela odorata* trees which prevent a richer and more abundant seed rain. Even with previous chili sowings and the intensive management of other crops, the low values recorded in Campeche may be the result of the high-water saturation of the Solonchak soil type during the rainy season; this saturation suffocates the reservoir seed bank and therefore the soil only contains newly arrived seeds (Castillo-Argüero *et al.*, 2002; Castellanos-Vargas *et al.*, 2017).

The floristic quantity present in each state was different. The cultivation fields where the soil samples were obtained had significant management differences: the samples from Yucatán and Campeche came from constantly cultivated land, while the crop from Quintana Roo was established on land which had a history of pasture and grazing management and had recently been opened to agricultural management. This can influence the number of families and species present in each sampling, as pointed out by Godoy *et al.* (1995) who indicate that the type of tillage modifies the floristic composition of the weeds that affect the crops by changing the vertical distribution of the seed bank.

The floristic diversity of each region changes according to the season, the year, and the climatic region. Many of these species are highly competitive and prolific, which turns them into reservoirs of both harmful fauna, as well as of beneficial insects (Kumar *et al.*, 2021). Families and species that favor the presence of insect that work as vectors of pathogens have been identified. Of the virus symptomatic plant samples from the seed collection sites, 31 were analyzed and the presence of a begomovirus was confirmed in 26 of them. The genera *Sida* (Malvaceae), *Rhynchosia* (Fabaceae), and *Corchorus* (Tiliaceae) were the most frequent, although specimens from other genera were also infected (Table 2).

Table 2. Weeds with begomovirus incidence in habanero chili (*Capsicum chinense*) cultivation sites.

Family	Genus	Symptoms	Begomovirus (*sensu lato*)
Malvaceae	*Sida* *Anoda* *Whalteria*	+ + +	+ + +
Convolvulacea	*Jacquemontia Merremia*	+ +	+ +
Fabaceae	*Rhinchosia Desmodium*	+ +	+ +
Tiliaceae	*Corchorus*	+	+
Cucurbitaceae	*Melothria*	+	+

The presence of begomoviruses in the weed species analyzed gives a perspective of their function as a source of inoculum for chili crops in these regions. Therefore, these species must be controlled before the crop is established. Begomoviruses transmitted mainly by *Bemisia* have been detected in species of the families Malvaceae, Euphorbiaceae, Asteraceae (Vaca-Vaca *et al*., 2019), Portulaceae, Amaranthaceae, and Tiliaceae (Bezerra *et al*., 2004; Waheed *et al*., 2016). In the study area it is common to find *Sida* sp., *Waltheria*, and *Euphorbia* plants infested by *Bemisia* and infected with begomovirus, although we must highlight that this is not the only insect of economic importance associated with weeds present in crops. Ávila-Alistac *et al*. (2017) report the preference of *Thrips tabaci* for *Ricinus communis* and *Acalypha ostryifolia* species, in the region of Michoacán, Mexico. This report is important because *T. tabaci* is a transmitter vector of the *Iris yellow spot virus* (IYSV). *Frankliniella occidentalis* is another economically relevant thrips, associated as a vector of the tomato spotted wilt virus (TSWV), which occurs more frequently in weeds of the Asteraceae family (Heinz-Castro *et al*., 2012).

Weed families reported as hosts of disease-transmitting insects were identified in the analyzed samples; however, it is necessary to understand the behavior of these insects and their weed-crop relationship (Capinera, 2005), in order to apply a sustainable management of the crop, avoiding excessive application of herbicides and insecticides.

CONCLUSIONS

The floristic diversity present in the cultivation of habanero chili is wide and varied in each state of the Yucatan Peninsula. The diversity present will depend on the cultivation practices, the time the land has been cultivated, the climatic condition, the type of soil, and the condition of the surrounding vegetation. Knowledge about the species present is necessary to propose improvements in technological packages and to allow a sustainable management.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Nitric acid and hydrogen peroxide in milk and cheese digestion for the detection of metals

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ABSTRACT

Objective: To determine the concentration of arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), and zinc (Zn) in milk and cheese by comparing the use of nitric acid (HNO_3) and a mixture of nitric acid and hydrogen peroxide ($HNO_3+H_2O_2$) in acid digestion.

Design/methodology/approach: The milk for the study was collected from storage tanks in various localities of Huejotzingo, and the different types of cheese came from Santa Ana Xalmimilulco, Huejotzingo, Puebla, Mexico. Digestion was carried out in a CEM MarsX microwave. Elemental concentrations were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).

Results: The combination of $HNO_3+H_2O_2$ in milk digestion resulted in higher concentrations of Cd, Pb, and Zn. Conversely, the concentration of As was higher when using only HNO_3 for digestion. No significant differences were found for Cr and Cu between the two digestion methods. In the case of Oaxaca cheese, digestion with HNO_3 resulted in higher concentrations of As. Acid digestions did not affect the concentrations of the remaining elements. The same behavior was observed for Ranchero cheese, since the digestion combining $HNO_3+H_2O_2$ resulted in lower As concentrations, as compared to the digestion with only HNO_3. Pb and Zn were not significantly affected by the treatments, while the concentrations of Cd, Cr, and Cu could not be determined when samples were treated with the $HNO_3+H_2O_2$ combination.

Limitations: In this work we were able to determine the concentration of a number of metals present in milk and cheese using nitric acid and hydrogen peroxide in acid digestion. However, we recommend more tests are carried out to establish which acid or combination of acids allows for a broader detection of metals.

Findings/Conclusions: According to the results obtained in this work, we can conclude that there is specificity for metal detection, with HNO_3 being the most efficient.

Keywords: Acid digestion, Metal contamination in milk, Cheese.

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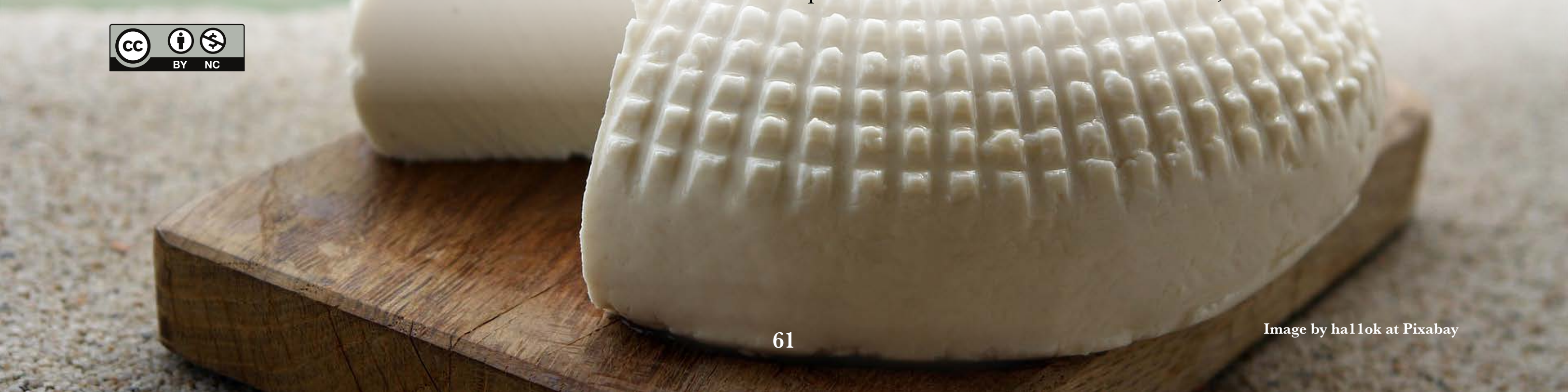
INTRODUCTION

Milk and dairy products are consumed by more than 6 million people in the world, most of whom live in developing countries. However, per capita consumption is higher in developed countries (FAO, 2020). This is because dairy products have a great nutritional value and are considered a complete and balanced food. Therefore, it should be a basic



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component in the diet of human beings, primarily for children due to its relation to linear growth and bone health (Fernández-Fernández *et al.*, 2015; Miedico *et al.*, 2016; Wallace *et al.*, 2020). Nevertheless, when milk is produced in contaminated environments, it can contain toxic substances and metallic elements that can affect its quality and consequently food safety and public health (Harlia *et al.*, 2018; Khaneghah *et al.*, 2020). The presence of elements such as lead (Pb), cadmium (Cd), zinc (Zn), chromium (Cr), and arsenic (As) has been reported in the milk of cattle that had ingested contaminated forage, water, and silage. Consequently, other dairy products may also contain toxic elements that come either from milk or from contamination during the production process. Evidently, this represents a health risk (Moreno-Rojas *et al.*, 2010; Çetinkaya *et al.*, 2016; Castro-González *et al.*, 2017; Castro-González *et al.*, 2018a; Zhou *et al.*, 2019).

Any toxic material contained in milk must be considered serious. Hence, metals contained in food, particularly in milk and its derivatives, constitute a highly relevant issue because they involve a broader contamination of the food chain, and because of the damages this can cause to public health (Mubbasher *et al.*, 2003; Ismail *et al.*, 2017b).

The consumption of metal-contaminated foods has caused various pathologies, such as neurotoxic damage, poor cognitive development, and inadequate organ development, which can lead to death from cardiovascular disorders, kidney failure, and different types of cancer (Dorea and Donangelo, 2006; Arora *et al.*, 2008; ATSDR, 2013; Ismail *et al.*, 2017a; Sujka *et al.*, 2019).

Metals enter milk primarily via the animal's ingestion of contaminated water and food (Castro-González *et al.*, 2018a). To quantify metal concentration, inductively coupled plasma optical emission spectroscopy (ICP-OES) has been used by various authors (Ríos-Arana *et al.*, 2004; Wang *et al.*, 2004; Bakircioglu *et al.*, 2011; Di Giuseppe *et al.*, 2014; Antunović *et al.*, 2018). To prepare the sample, it must be digested in order to destroy the organic matter and obtain a complete solution of analytes. This will allow to conduct the analysis with the greatest possible accuracy (Ayala-Armijos and Romero-Bonilla, 2013). The digestion procedure may have variations that influence the quantification of metals for specific components. It is therefore necessary to assess different methods and find those that will effectively identify the presence of these toxic elements. This in turn will lead to results that allow for control strategizing and risk mitigation.

The town of Santa Ana Xalmimilulco, Huejotzingo, Puebla, Mexico, concentrates a daily production of approximately 45 tons of raw milk —part of which is used to make cheese— from different neighboring communities. Here, animals used for production are fed with forages that are grown in soils irrigated with wastewater containing discharges of different industries located in the area, among them a textile industrial park, the Quetzalcóatl industrial park, and a petrochemical plant.

The objective of this research was to determine the concentration of metals —arsenic (As), cadmium (Cd), copper (Cu), chromium (Cr), lead (Pb), and zinc (Zn)— in raw milk and in two types of cheese, Oaxaca and Ranchero, derived from it, comparing the use of nitric acid and of a combination of nitric acid and hydrogen peroxide in the acid digestion process.

MATERIALS AND METHODS

Location of milk and cheese collection points

The work was carried out in the summer of 2019, in the municipality of Huejotzingo, in the state of Puebla, Mexico (19.12° 30.81' N and 98.24° 36.78' W), at an altitude of 2,228 m (Figure 1).

Sampling and sample transport

Milk samples were collected randomly from milk storage tanks at the different localities studied (Figure 1). This milk comes from small production units, where cattle is fed with forages that are grown in soils irrigated with wastewater. Meanwhile, both types of cheese were acquired from the factory with the largest cheese production in the region, located in Santa Ana Xalmimilulco, Puebla.

Ten 100 g cheese samples were collected —five of Oaxaca cheese and five of Ranchero cheese— every week for five weeks. Milk samples were collected using 50 mL Falcon tubes (Fisher Scientific, Waltham, MN, USA) previously treated according to the methodology described by Castro-González *et al*. (2018a). All samples were transferred in a refrigerator at a temperature of 4 °C to the laboratory, where they were frozen at −80 °C. Subsequently, all samples were lyophilized using a LABCONCO lyophilizer (FreeZone 4.5 Liter Benchtop, Kansas City, MO, USA).

Sample digestion

The milk and cheese samples were digested in a microwave oven (CEM MarsX, CEM Corporation, Mathews, NC, USA). We took 0.5 g samples of the lyophilized matrices for subsequent digestion. We carried out two variants of the digestion process per sample and per matrix. For the first one we used 10 mL of HNO_3 and for the second one, 5 mL of HNO_3 and 5 mL of H_2O_2. Nitric acid was of high purity (65%; Merck, Darmstadt, Germany), while H_2O_2 was 30% v/v (Merck, Darmstadt, Germany). In both cases, the

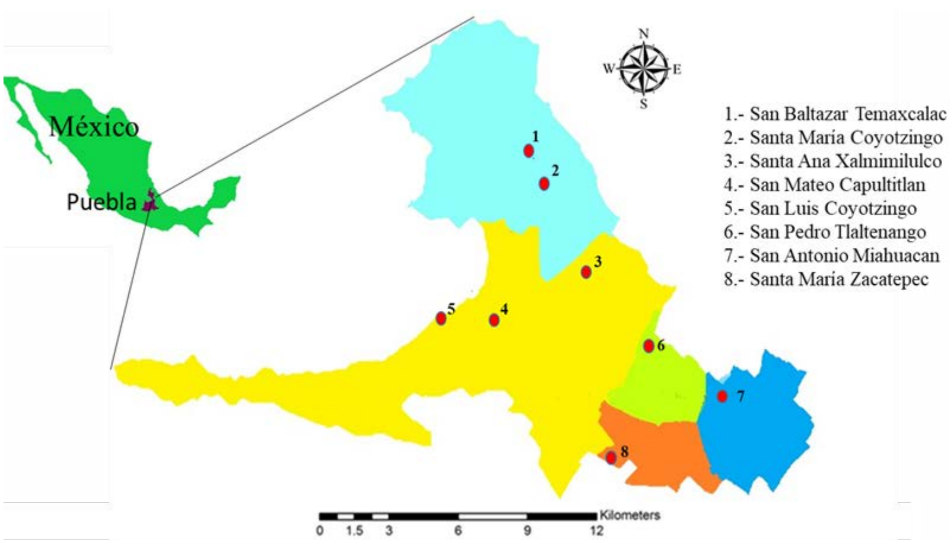

Figure 1. Location of milk collection centers in the municipality of Huejotzingo, Puebla, Mexico.

samples were placed in the microwave at 1600 W power, with a ramp time of 15 min to reach a temperature of 200 °C at a pressure of 800 psi and a holding time of 15 min. Once digested, the samples were filtered using Whatman grade 42 paper (GE Healthcare, Little Chalfont, UK), diluted to 50 mL with deionized water, and left cooling at 3 °C until analysis.

Analyte quantification

The concentrations of As, Cd, Cu, Cr, Pb, and Zn were determined using inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian 730, Mulgrave, Victoria, Australia). The solutions were prepared in type I deionized water (18.2 MΩ cm^{-1}). All chemicals used were analytical reagent grade. Calibration standards were prepared using an ICP multi-element standard solution XVI (Merck, Darmstadt, Germany).

Precision and accuracy levels (Table 1) were established using five targets and ten repetitions. Quality control was carried out with a standard and a control sample, which was applied after every 20 samples analyzed. The method described by Khan *et al.* (2014) was used to determine the limit of detection (LOD) and the limit of quantification (LOQ).

The experimental design was completely randomized. First, data were subjected to the Lilliefors (Kolmogorov-Smirnov) normality test, establishing a value of $\alpha=0.05$. Subsequently, a non-parametric analysis of variance was carried out: the Kruskal-Wallis test. To determine the significant differences between matrices, the Kruskal-Wallis post-hoc test was applied for each metal, using software R version 4.0.4 R Commander.

RESULTS AND DISCUSSION

Table 2 presents the concentration of metals found in milk samples, while Table 3 shows the concentrations found in Oaxaca and Ranchero cheeses.

Milk

The combination of nitric acid and hydrogen peroxide ($HNO_3+H_2O_2$) for milk digestion resulted in higher concentrations of Cd, Pb, and Zn. Conversely, the concentration of As was higher when using only nitric acid (HNO_3). In the case of Cr and Cu, no significant differences were found between the two types of digestions.

Table 1. Precision and accuracy levels and quality control of the milk and cheese samples analyzed.

Standards	Elements					
	As	Cd	Cr	Cu	Pb	Zn
RDS (%)	16.84	12.59	3.19	4.58	14.54	1.66
LOD (mg L^{-1})	0.01	0.001	0.001	0.002	0.008	0.003
LOQ (mg L^{-1})	0.03	0.004	0.002	0.007	0.03	0.009
r^2	0.999	0.999	0.999	0.999	0.999	0.999
Vr (%)	104	106	106	99	101	103

RDS=relative standard deviation; LOD=minimum detectable quantity; LOQ=minimum quantifiable quantity of equipment; r^2=correlation coefficient; Vr=recovery value.

Table 2. Metal concentrations (mg kg^{-1}) in milk, after digestion with nitric acid (HNO_3) and a mixture of nitric acid and hydrogen peroxide ($HNO_3+H_2O_2$).

Metals	**Milk digestion**	
	HNO_3 (mg kg^{-1})	**$HNO_3+H_2O_2$ (mg kg^{-1})**
Cd	0.001±0.001 b	0.010±0.006 a
Pb	0.026±0.013 b	0.035±0.016 a
As	0.130±0.070a	0.080±0.080 b
Cr	0.028±0.003 a	0.024±0.020 a
Cu	0.014±0.004 a	0.016±0.007 a
Zn	0.540±0.080 b	0.740±0.090 a

Means±SD with different letters within each row indicate significant differences ($p \leq 0.05$).

Table 3. Metal concentrations (mg kg^{-1}) in Oaxaca and Ranchero cheeses, after digestion with nitric acid (HNO_3) and a mixture of nitric acid and hydrogen peroxide ($HNO_3+H_2O_2$).

Metals	**Oaxaca cheese**		**Ranchero cheese**	
	HNO_3 (mg kg^{-1})	**$HNO_3+H_2O_2$ (mg kg^{-1})**	**HNO_3 (mg kg^{-1})**	**$HNO_3+H_2O_2$ (mg kg^{-1})**
Cd	0.001±0.001a	ND	0.003±0.001 a	ND
Pb	0.060±0.05 a	0.08±0.03 a	0.110±0.040 a	0.11±0.04 a
As	0.180±0. 110a	0.05±0.07 b	0.160±0.070 a	0.05±0.07 b
Cr	0.010±0.003 a	ND	0.020±0.020 a	ND
Cu	0.010±0.006 a	ND	0.018±0.010a	ND
Zn	0.710±0.180 a	0.84±0.19 a	0.240±0.100 a	0.36±0.2 a

Means±SD with different letters within each row indicate significant differences ($p \leq 0.05$).

Cheeses

Digestion with HNO_3 resulted in higher concentrations of As in the case of Oaxaca cheese. The remaining elements were not affected by either of the treatments. The same behavior was observed in the case of Ranchero cheese, since digestion with $HNO_3+H_2O_2$ resulted in lower As concentrations, as compared to digestion with only HNO_3. Pb and Zn were not significantly affected by either of the treatments, while the concentrations of Cd, Cr and Cu could not be determined when samples were treated with the $HNO_3+H_2O_2$ mixture.

Previous studies have reported As levels in milk below the values found in this work when HNO_3 was used for digestion. For milk produced in La Laguna region of Mexico, for instance, Rosas *et al.* (1999) found As values of 27.4 ng g^{-1} handling a combination of HNO_3, H_2O_2, H_2SO_4, and HCl. In Italy, Licata *et al.* (2004) reported 0.04 mg kg^{-1} of As, using HNO_3 and H_2O_2 (5:2) with atomic absorption spectroscopy. Castro-González *et al.* (2017) evaluated As in milk using HNO_3 for digestion in an area irrigated with wastewater in Mexico and reported 0.034 mg kg^{-1} using inductively coupled plasma optical emission spectroscopy.

The value obtained for As (0.13 mg kg^{-1}) in milk when using HNO_3 in digestion is close to the maximum of 0.15 mg kg^{-1} determined by the WHO-FAO (Codex Alimentarius Commission, 1995) and the corresponding European Union regulation (DOUE, 2006), and below the maximum of 0.2 mg kg^{-1} indicated by the Official Mexican Standard (SSA, 2010).

Taking the detected value of As into consideration is very important, since high concentrations of this element can cause carcinogenic effects by increasing oxidative stress, direct genotoxicity, altered DNA repair, and growth factor expression (Ghosh and Sil, 2015).

Milk digestion with H_2O_2 allows for a better detection of Cd, Pb, and Zn. In the case of Cd, this process evinced a value of 0.01 mg kg^{-1}, which is higher than the 0.001 mg kg^{-1} reported in Mexico by Castro-González *et al.* (2017), who used HNO_3 for digestion, and by Najarnezhad and Akbarabadi (2013) in Iran, who used a mixture of HNO_3+H_2O_2. The value of Cd found in this work is below the 0.10 mg kg^{-1} maximum threshold set by the MERCOSUR Technical Regulation (2011). In Mexico, the Official Mexican Standard (SSA, 2010) does not establish a maximum value for Cd in milk. The European Union regulation (DOUE, 2014) establishes maximum limits for Cd in various food products, including cereals, vegetables, meat, fish, shellfish, offal, and food supplements, but it does not do so in the case of milk and milk by-products. The presence of this metal in milk is important, since it brings no benefits to human metabolism, is considered extremely toxic, and has carcinogenic effects (IARC, 2012).

In the case of Pb, we observed that although values for both types of digestion were significantly different, in both cases they exceeded the maximum level of 0.020 mg kg^{-1} for milk according to CODEX (Codex Alimentarius, 1995) and the standards of the European Union (DOUE, 2015; UE, 2017). However, both values were below the maximum of 0.1 mg kg^{-1} set by the Official Mexican Standard (SSA, 2010), and of 0.046 mg kg^{-1} reported by Castro-González *et al.* (2017) using HNO_3 for digestion. It must be noted that these values are averaged over two seasons, unlike the values found in this work, which were limited to summer. The value found in this work with the mixture of HNO_3+H_2O_2 is higher when compared to that reported by Najarnezhad and Akbarabadi (2013), set at 0.008 mg kg^{-1} using 4 mL of HNO_3 and 4 mL of HNO_3+H_2O_2 per sample for digestion.

The results obtained for Zn in milk are below the values (4.96 mg kg^{-1}) reported by Semaghiul *et al.* (2008) using HNO_3, but they are higher for both digestions (Table 2) than those reported by Castro-González *et al.* (2017) (0.44 mg kg^{-1}) in Mexico using HNO_3.

The average value we found for copper was of 0.016 mg Cu kg^{-1}, which was higher than that reported by Patra *et al.* (2008), set at 0.002 mg kg^{-1} using a combination of HNO_3:$HClO_3$ (4:1; v:v) for digestion, but lower than that reported by Castro-González *et al.* (2017), set at 0.029 mg kg^{-1} using only HNO_3. The concentrations of Cu found for both digestions in milk (0.014 and 0.016 mg kg^{-1}) (Table 2) are lower than those established by Bilandžić *et al.* (2011), of 0.937 mg kg^{-1}, and Malhat *et al.* (2012), of 2.8 mg kg^{-1}.

In both types of cheese, concentrations of As (0.16 mg kg^{-1}) and Pb (0.11 mg kg^{-1}) are below the maximum established by the Official Mexican Standard (SSA, 2010), set at 0.1 mg kg^{-1} and 0.2 mg kg^{-1}, respectively. However, they are higher than the concentrations reported by Castro-González *et al.* (2018b): 0.05 mg kg^{-1} for As and 0.17 mg kg^{-1} for Pb. Moreno-Rojas *et al.* (2010) reported Pb values of 0.013 mg kg^{-1} for Afuega'l pitu cheese and 0.026 mg kg^{-1} for Pasiego cheese, which are below the results obtained for Oaxaca and Ranchero cheeses regardless of the digestion method. Meshref *et al.* (2014) found 0.47 mg P kg^{-1} for Kareish cheese, which is above the value determined in this work for Oaxaca and Ranchero cheeses. However, these authors used a combination of HNO_3: $HClO_3$ (4:1; v:v) for digestion, and flame atomic absorption spectroscopy to perform the metal analysis. Christophoridis *et al.* (2019) reported values of 15.3 μg Pb kg^{-1} for Metsovone cheese and of 12.1 μg Pb kg^{-1} for Cream cheese, which are below the concentrations found in this work. The maximum value for cheese should be compared to the maximum established by milk standards, since cheese is the product of milk coagulation.

Values detected for cadmium using only HNO_3 for digestion were 0.001 mg Cd kg^{-1} for Oaxaca cheese, and 0.003 mg kg^{-1} for Ranchero cheese. Said values are below those found by Yüzbasi *et al.* (2009), set at 0.004 mg Cd kg^{-1} for fresh cheese using HNO_3 for digestion.

The detected values of 0.11 mg Pb kg^{-1} and 0.003 mg Cd kg^{-1} in our study for the case of Ranchero cheese are below the values reported in the case of Turkish Cami Bogazi cheese by Çetinkaya *et al.* (2016), established at 0.18 mg Pb kg^{-1} and 0.028 mg Cd kg^{-1}, respectively, using $HNO_3+H_2O_2$ for digestion in the same proportions as those used in this work. These authors also reported 2.75 Zn mg kg^{-1}, which is higher than the values detected in this research for both cheeses and both types of digestion.

The fact that element detection was better when using HNO_3 may be due to the amount used, since it reduces hydroscopic capacity and increases oxidation in the mixture to be analyzed. Also, it is known that nitrates allow for better readings. However, the detection of some metals requires the addition of other compounds, such as the H_2O_2 used in this work. Nevertheless, mixing compounds can interfere with the detection of some metals, probably because digestion might not be strong enough to provide an efficient detection.

CONCLUSIONS

According to the results obtained, we conclude that there is specificity for the detection of some metals, nitric acid being more effective in detecting As in digested milk. Meanwhile, the combination of nitric acid and hydrogen peroxide is more efficient for the detection of Cd, Pb, and Zn. In the case of cheese analysis, nitric acid is stronger for digestion and better for the detection of Cd, As, Cr, and Cu. It is important to continue testing different reagents to be even more accurate in detecting these elements in food, due to the danger they pose to health and food safety.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Infestation assessment with *Haematobia irritans* in grazing cattle and stress behaviors in tropical regions

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ABSTRACT

Objective: To evaluate the infestation with flies in grazing cattle, and its relationship with some behaviors (tail butting, head butting, kicking and rubbing) that alter animal welfare, through direct observation and use of photographs.

Design/Methodology/Approach: At two times (7:00 and 14:00 h) the variables were measured on thirty naturally infested cows and randomly distributed in two treatments: TS: control without deworming and TD: chemically dewormed.

Results: The fly infestation were higher ($p<0.001$) in TS cows (483.7 flies/animal), they also, expressed with greater intensity ($p<0.001$) and frequency of upset behaviors: tail-tapping (10.84 movements min^{-1}), head-butting (1.66), kicking (0.51) and rubbing (0.33) in order to drive away the annoying contact and aggression of the ectoparasite.

Limitations: More in deep research is needed in order to assess the physiological disorders that this parasite could cause by altering well-being of grazing cattle in the tropics.

Findings/Conclusions: It is concluded that the greater the fly infestation, the movements that alter the welfare of the animals' increase; however, more research is required to know the physiological welfare consequences that the infestation of this parasite implies.

Keywords: Hun flies, dual purpose cattle, ectoparasites, well-being, stress behaviors

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INTRODUCTION

Ectoparasites associated with livestock are a major concern worldwide due to their economic, health and welfare impacts, which can be direct through tissue damage and blood loss or indirect due to their role as vectors of viral, bacterial, protozoan and helminths pathogens (Trout-Frixell *et al.*, 2021). A second category of indirect effects are

those that result from the alteration of the behavior of cattle induced by the attack of ectoparasites (Eiras *et al*., 2021). Effective control is challenging and relies primarily on the use of chemical insecticides and miticides (Sarwar and Arfa, 2018; Madhav *et al*., 2020). The horn fly (*Haematobia irritans*) is a natural hematophagous ectoparasite of cattle, especially grazing. Adult flies spend most of their lives attached to cattle, they tend to congregate on the back and shoulders or on their belly and legs during the hottest hours of the day (Almazán-García *et al*., 2001; Pérez de León *et al*., 2020). Flies usually feed 20 to 30 times a day, they only separate from their host to oviposit. Cattle infested with 200 flies have been reported to cause a loss of 520 mL of milk per day and 28 g of live weight per animal per day (Fuentes-Castillo *et al*., 2016). In tropical livestock, the fly is present throughout the year, with greater abundance in the warmer and humid months from August to October (Cruz *et al*., 2000; Galindo-Velazco *et al*., 2008) and provoke stress behaviors in cattle, such as movements of ears, head blows, kicks, skin movement, muscle contractions, tail movements, licks, in order to ward off flies attached to the body and reduce discomfort, generating a greater expenditure of energy and changes in your eating habits normal (Cruz *et al*., 2000; Almazán-García *et al*., 2001; Vitela-Mendoza *et al*., 2016; Barragán-Hernández *et al*., 2019). The way described to measure the degree of infestation is to count the number of flies by direct observation (Cruz *et al*., 2000; Alonso-Díaz *et al*., 2007; Galindo-Velazco *et al*., 2008; Fuentes-Castillo *et al*., 2016; Vitela-Mendoza *et al*., 2016). However, under grazing conditions it is a determine challenge the flies number by visual counting, mainly when the density is high, since the flies have the ability to fly easily, adhere to another part of the same animal or land on other animals in only seconds, also the movement of cattle to keep flies away, are factors that interrupt the count (Smythe *et al*., 2017; 2020). It is essential in animal welfare evaluations to have an easy and fast method to measure the number of flies adhering to the body of animals. The objective was to evaluate, through direct observation and the use of photographs, the degree of flies' infestation on grazing cattle, and its relationship with some behaviors (number of tail butting, head butting, kicking, rubbing and flies count) that alter animal welfare.

MATERIALS AND METHODS

Study area

The study was carried out in Guerrero, Mexico (18° 25' NL and 100° 43' WL), during the rainy season (July). The climate is considered hot dry (Aw0) with rains in summer, with a temperature between 36 to 39 °C and an average relative humidity of 85%. Annual rainfall of 750 mm (June to September) and an altitude of 250 m.

Production units

The study was developed in six production units with a semi-extensive system and continuous grazing (day and night) in mixed native grasslands with Bermuda grass (*Cynodon dactylon*), purple nutsedge (*Cyperus rotundus*) and muhly grass (*Muhlenbergia macroura*). The herd structure was between 20 and 40 animals of heterogeneous ages and sex (young, adult, male and female). The racial compositions were hybrid animals *Bos indicus* × *Bos taurus* (Brahaman, Gyr, Sardo negro × Brown Swiss, Simmental, Beefmaster, Charolais). In each production unit, 5 cows with dull fur were randomly selected, accustomed to aflight zone between 1 and 2 m away.

Experimental design

The animals of each ranch were randomly distributed in two treatments, one control without deworming (TS) and another experimental subject to an external deworming scheme (TD: alternate baths every three days by spraying with 15% cypermethrin at a dose of 1 mL L^{-1} and chlorpyrifos at 24% at doses of 1 mL L^{-1} during the 36 days of the evaluations), in this way each treatment had 15 cows as experimental units, on which the measurement of the variables was developed.

Variables measured

The study lasted 36 days, in all animals the variables were measured and recorded by the same evaluator under the same criteria, through direct observation and the use of photography (Table 1). The variables were evaluated in repeated measures for six days at two times 7:00 and 14:00 h for ten minutes in each cow. Five cows of a ranch per day at a time, in the grazing areas.

The degree of fly infestation was evaluated by counting the number of flies adhering to the body of the animal: scapula-back, legs, belly (lateral and lower part) and neck of one side of the animal and the result was multiplied by two to obtain the total number of flies per animal, at the same time photographs were captured to relate the images with the counts of the ectoparasite. During the observation time, the number of times that the animals performed some behavior related to the attempt to drive away or repel the flies from their body was also recorded as described in Table 1

Analysis of data

The data of the variables of each treatment were analyzed using the Man Whitney non-parametric test and a minimum significance of 0.05 was used. In addition, a Pearson correlation analysis was developed considering the degree of infestation by flies as an independent variable and as dependent variables the number of behaviors (tail butting, head butting, kicking and body rubbing) developed by the cows.

Table 1. Cattle movements related to the attempt to ward off or repel aggression or infestation by horn flies (*Haematobia irritans*).

Behaviors	Description
Pigtails	The tail movements were considered when it hit the side of the animal or exceeded the back of the animals in order to keep flies away.
Heading	The times that the animals performed head movements towards the back or belly to ward off the flies were counted.
Kicks	The movements that the animals performed with their hind legs to keep flies away from the lower part of their belly were considered.
Rub	The number of times the animals rubbed some part of their body with bushes, stems or tree branches was counted.

RESULTS AND DISCUSSION

The results firstly show that any scientifically proven control method can help to reduce the infestation of flies in cattle. Figure 1 shows that the degree of infestation in cows with deworming (TD) was low, while in cows without deworming (TS), oscillating from moderate to high and with a higher incidence the extreme.

The number of flies adhering to the body surface of the cows was higher ($p \leq 0.05$) in the non-dewormed animals (TS) compared to the dewormed cows (TD) (Figure 2). Also linearly, the cows of the TS that had a higher degree of infestation were those that developed more behaviors to repel flies ($p \leq 0.05$) and of these the tail movement was the most predominant (108.04 movements cow^{-1} 10 min^{-1}), followed by head butting (16.65 movements cow^{-1} 10 min^{-1}), kicking (5.17 movements cow^{-1} 10 min^{-1}) and rubbing their body (0.33 movements cow^{-1} 10 min^{-1}), compared to those expressed by TD cows, respectively (Figure 3). These observations show that the attack of flies on grazing cattle is uncomfortable and annoying for the animals, which causes stress and additional energy expenditure, in addition, the presence of these vectors increases the risk of diseases, and all this deteriorates the conditions of animal welfare. Mullens *et al.* (2017) reported that the main defensive behaviors exhibited by fly-infested cattle were head throws, leg strikes, panic reflex and tail flick. The tail is the part of the body that animals use the most to temporarily drive away flies and it is likely that they do so because it is a part that they can move more easily to easily reach a large part of the body where the flies. The head movement was the second most performed by the animals, this movement requires more effort and the animals only performed it when the intensity of discomfort was greater, including at the same time the movement of the ears and lick. The movement of legs (kicks)

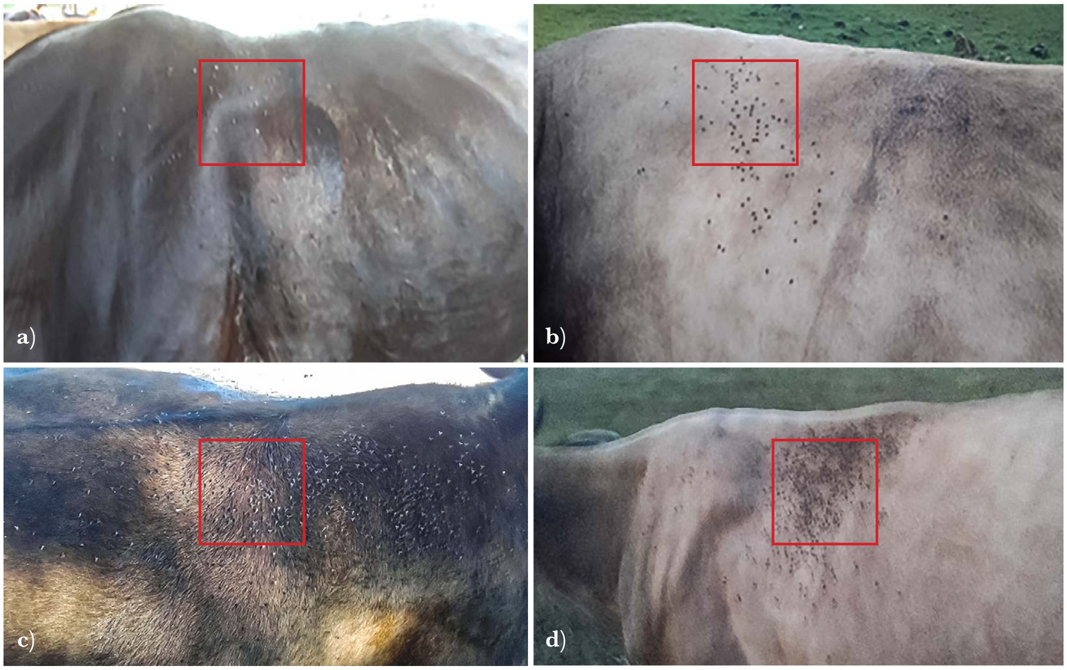

Figure 1. Horn fly (*Haematobia irritans*) infestation degrees observed in the cows with deworming (TD) (low: a) and cows without deworming (TS): moderate (b), high (c) and extreme (d).

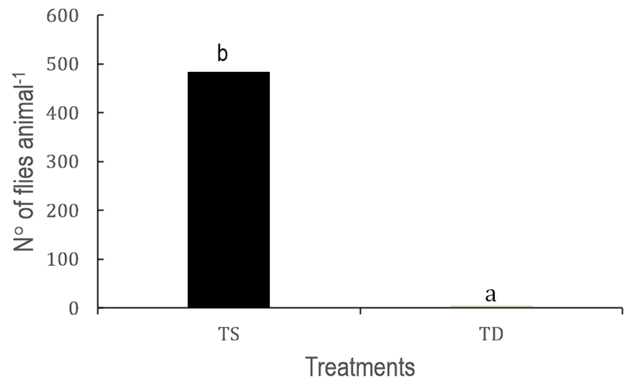

Figure 2. Number of flies per animal in non-dewormed animals (TS) and dewormed cows (TD) grazing under tropical conditions. Means with different literal indicates statistical difference (p≤0.05, Mann-Whitney test).

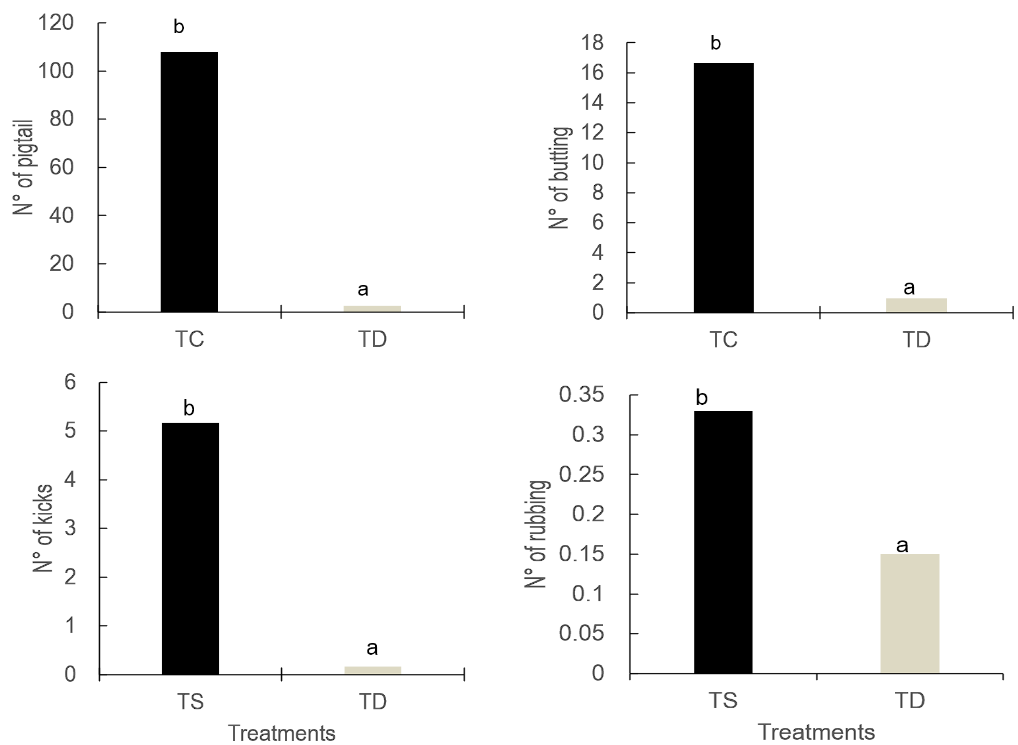

Figure 3. Movements number of non-dewormed (TS) and dewormed cows (TD) grazing under tropical conditions. Means with different literal indicates statistical difference (p≤0.05, Mann-Whitney test).

is carried out when the flies are attached to the lower part of the belly and the extremities. In addition to the body movements that animals use to keep flies away, resort to other alternatives such as rubbing your body on the branches of shrubs or the stem of trees. The number of behaviors performed by the animals during the observation period were compared to those obtained by Kojima *et al.* (2019) who reported a total of 54 movements in cow and an infestation of 120 flies, and also positively related the number of flies with the stress behaviors developed by the animals.

The number of flies was different ($p \leq 0.05$) with 483.77 and 5.51 flies cow^{-1} for TS and TD, respectively (Figure 2). The infestation of TS cows is classified as high within the standard of Smythe *et al.* (2017) which considers low level 0, medium 250, high 500 and extreme 1000 flies. It was also confirmed that flies are responsible for affecting the behavior and welfare of livestock. The numbers of flies were higher during the morning count (07:00 h) on both sides of the back of the animals. The count at 14:00 h registered a lower amount of the parasite perched on the body of the animals, in the ventral body regions (belly, legs and chest) seeking shelter from the sun's rays (Smythe *et al.*, 2020), which made counting difficult at these hours. The quantities of flies/cow obtained are much higher than those reported by other authors such as Kojima *et al.* (2019) with 120 flies cow^{-1}, and Vitela-Mendoza *et al.* (2016) with 55 flies cow^{-1}, while Galindo-Velazco *et al.* (2008) reported three peaks during the year with values of 156, 236 and 120 flies animal^{-1}. Fuentes-Castillo *et al.* (2016) found infestations of 50 to 56 flies animal^{-1} over the course of the year. Almazán-García *et al.* (2001) reported maximum values during the year of 200 flies animal^{-1}.

Correlation between the infestation degree and stress behaviors

The correlation between the number of behaviors developed by the animals and the number of flies adhering to their bodies was significant. Table 2 shows that the number of tail butting, head butting, kicking and body rubbing against bushes and other animals increased significantly and gradually with the degree of fly infestation with a correlation value of 0.98 ($p \leq 0.05$), 0.96 ($p \leq 0.05$), 0.95 ($p \leq 0.05$) and 0.89 ($p \leq 0.05$), respectively.

The correlation analysis showed that the flies altered the normal behavior and well-being of the animals by causing body movements to repel and drive away the ectoparasite. Also, Trout-Frixell *et al.* (2021) observed that the behaviors developed by cattle to ward off flies are related to the intensity of the infestation. The images show that when the number of flies is lower, they adhere separately on the back of the animal, mainly during the cooler hours of the day (07:000-09:00 h) and when the density is high, they are placed at distances shorter between them (Figure 1a-d).

Smythe *et al.* (2017) they suggest that digital photographs taken of infested cattle provide estimates that are just as accurate as traditional visual counts. However, they argue that more research is needed to standardize this technique. Mochi *et al.* (2009) and Mullens *et al.* (2016) recommended the use of high-resolution digital cameras to capture images

Table 2. Correlation of the infestation degree (flies' number) by horn flies (*Haematobia irritans*) with the behaviors that reflected stress in grazing cattle in a tropical environment.

Behaviors	Flies number				Correlation
	0-100	101-200	201-400	>401	
Pigtail	5.9	70	147	214	0.98*
Butting	1.7	10	20	32	0.95*
Kicks	0.3	5.5	7.5	7.7	0.96*
Rubbing	0.3	0.4	0.5	0.8	0.88*

*$p \leq 0.05$

of infested cattle and improve the practicality of counting. Unfortunately, this method is not without its challenges since avoidance behaviors of cattle and human interaction make it difficult to obtain images clear and reliable. Smythe *et al.* (2020) indicated that deep thinking, computer vision, and object detection frames can be adapted for future fly counting. Trout-Frixell *et al.* (2021) mentioned that the estimation of face flies in horses is carried out by producers through direct observation, counts and by lesions observed in the animals' eyes.

Recommendations for the reliability of the technique

It is suggested that the evaluation of the fly infestation on the animals is carried out by observation during the hours of less solar radiation (morning or afternoon). It is important to carry out the evaluation in 10% of mainly adult animals, with dark fur and that allow human approach at short distances to favor observation. Observe the lateral dorsal part of either side of the animal to classify the observation in one of the categories low, moderate, high or extreme. Considering the low category as a normal density that animals can support without compromising their welfare. The moderate category can be considered as the limit threshold that the animals can withstand, from this density the animals express a lack of well-being and decrease in production. In the high and extreme categories, the welfare of the animals is null and obviously the production is affected considerably.

CONCLUSION

Visual observation can be used as a viable alternative to estimate the infestation by the horn flies (*Haematobia irritans*) adhering to cattle grazing under tropical conditions to classify the degree of infestation of the animals. The intensity of the behavioral movements that grazing cattle perform to keep flies away from their bodies are related to the number of flies attached. It is important to consider more research in this regard that allows us to obtain results that assess the physiological disorders that this parasite could cause by altering well-being.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of Enerplant® doses on the development and nutrient use by cacao (*Theobroma cacao* L.) seedlings

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ABSTRACT

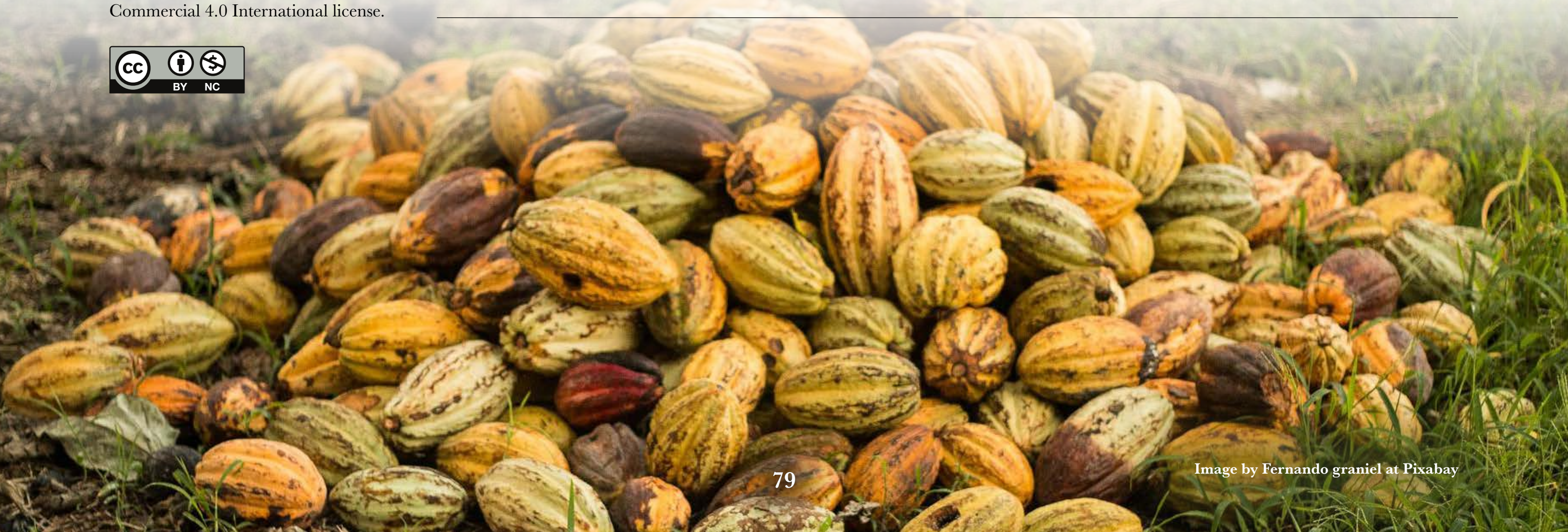
Objective: To evaluate the effect of the application of biostimulant doses on the development, absorption, and use efficiency of nitrogen, phosphorus, and potassium by cacao seedlings.

Design/Methodology/Approach: Five doses of Enerplant® (0, 1, 2, 3, and 4 mL ha^{-1}) were applied to cacao seedlings grown under a saran mesh. The study was carried out using a completely randomized design. The foliar area received a monthly application of the biostimulant. In order to determine the nutrient absorption, 3 plants per treatment were divided into leaves, stems, and roots; they were subsequently dried for 72 h at 65 °C. Finally, they were ground, packaged, labeled, and sent to the laboratory, where the macronutrients of each component were analyzed.

Results: The cacao seedlings had a positive response to the biostimulant application. In average, the results of our two-year study were: 1 mL ha^{-1} doses of Enerplant® significantly increased height (24%), dry mass (30%), quality index (58%), and foliar area (56%) of the cacao seedlings. Doses higher than 1- and 2-mL ha^{-1} decreased plant growth. The nutrient absorption by cacao in the nursery stage followed the following pattern: K>N>P.

Findings/Conclusions: The efficiency of nutrient use —regardless of the Enerplant® dose applied— was higher for P, followed by N and, finally, K. Applying 2 mL ha^{-1} doses of the biostimulant guaranteed the highest absorption and use efficiency values of macronutrients by cacao seedlings.

Key words: biostimulant, efficiency, nutrition, *Theobroma*.



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INTRODUCTION

In Cuba, most cacao crops are located in the eastern region of the country. In order to maintain and increase the productive levels of cacao, Cuba has developed specific programs for the various companies involved. In these programs, complying with seedling production plans plays a major role.

Cuenca-Cuenca *et al.* (2019) carried out a summary of the researches about the positive effects of mineral fertilization on cacao seedlings, the edaphoclimatic conditions influence, the responses to each nutrient, and the different fertilizers doses. These authors suggest determining the efficient nutrient use for cacao clones and taking into consideration their interaction with the specific growing area.

In Cuba, there is a lack of literature about the nutrimental requirements of cacao in the nursery stage. In Peru, researches determined that cacao plants in greenhouses (2-6 months old) require 2.4 kg N ha^{-1}, 0.6 kg P ha^{-1}, and 2.4 kg K ha^{-1} (García, 2018). Based on this information, a calculation of the costs required to meet the mineral fertilizer demand could be made —including the currently high costs of the said fertilizers. In view of this situation, a search for alternatives aimed to guarantee cacao nutrition should be carried out. Biofertilizers and biostimulants are one of these alternatives. The market for these products has seen a remarkable increase throughout the world (M&M, 2019).

Biostimulants are organic-based substances, which include vegetable regulators, as well as other substances that promote an indirect vegetal growth (such as carbohydrates and aminoacids) (Galindo *et al.*, 2019). Kumar and Aloke (2020) provide other definitions, including the mandatory need to have their positive effects proven by scientific institutions; those effects should stimulate nutrient absorption, efficiency, abiotic stress tolerance, and harvest quality. Meanwhile, Rouphael and Colla (2020) mentioned the biostimulant effects, taking into account physiological and agronomic aspects, as well as the stress tolerance, and an increase in the efficient use of nutrients.

Enerplant® is a growth stimulating product of vegetable origin, produced by the Mexican company Biotec International S.A. de C.V. This product optimizes the macro and micronutrient assimilation, intensifies the growth, development, and fruit formation processes, offers significant production increases, and a higher disease and extreme temperature resistance (Biotec Internacional, 1996). Its chemical composition includes an active ingredient made up of an oligosaccharide mix (0.01%) and an inert ingredient made up of dextrose (80%), maltodextrine (19.68%), citric acid (0.15%), and a coloring agent (0.17%) (Noriega, 2009). Enerplant® is an organic agriculture certified product.

In Cuba, Cobas-Elías *et al.* (2016) recorded an increase of sugarcane yield when a 100% of SERFE+5.2 mL Enerplant® ha^{-1} treatment was used; meanwhile, Núñez-Chávez *et al.* (2019) pointed out that an increase in the industrial yield of the spring remaining, ratooning, and sprout was obtained using a 2.6 mL ha^{-1} Enerplant® dose and 4 mL ha^{-1} of FitoMas-E. Alarcón *et al.* (2018) reported that using a 1.3 mL ha^{-1} dose increased onion yields and bulb quality. Finally, while studying three biostimulant doses in lettuce, Baldoquin-Hernández *et al.* (2015) determined that a 1.5 mL ha^{-1} dose had economic benefits.

There are few works about biostimulant use on coffee seedling grown in Brazil (Ferreira *et al.*, 2018); however, researches about the use of these products on coffee are available in Cuba (Bustamante and Ferrás, 2019). Nevertheless, this type of studies does not include cacao.

Therefore, a research aimed to establish the effect of Enerplant® doses on the development, foliar content, absorption, and use efficiency of N, P, and K by *Theobroma cacao* L. seedlings was carried out.

MATERIALS AND METHODS

The research was carried out in the Estación Experimental Agro-Forestal del Tercer Frente nursery (20° 09' N and 76° 16' W, at 135 masl), Santiago de Cuba province, during two periods: January-May 2019, and February-June 2020.

During the first and second experimental periods, the average temperature was 24.5 °C and 25.9 °C, respectively. The average rainfall was 473.5 mm in 33 days, and 265.5 mm in 32 days, respectively.

The experiment was developed in a completely randomized design, where the effect of five Enerplant® doses (0, 1, 2, 3, and 4 mL ha^{-1}) on seedlings of the *Theobroma cacao* L. UF 650 clon was studied. The seedlings were obtained from the hybrid seed bank of the institution.

Enerplant® was supplied by Biotech Internacional©. The product was monthly applied on the foliar area, before 10 am, from the second to the fifth leave, using a 16-L Matabi sprayer, at a constant pressure.

Metal divisions were used to avoid interferences between the plots during the application. Control was watered on the same day as the rest of the treatments.

The seeds were sown in 12.5×25 cm black polyethylene bags. Each treatment was comprised of 34 plants; each plant was an experimental unit. Fifteen experimental units per treatment were used in the analysis.

In order to control bright sunshine, a black saran mesh was used. The mesh allowed 50% of the sunlight to pass through and was placed above the bags, as well as in one side of the greenhouse, in order to protect plants from direct radiation.

The plants were grown in a substrate (brown soil without carbonate/cattle manure, in a 3:1 ratio) with slightly acid pH values, high organic matter content, available phosphorus and potassium levels, and normal exchangeable cation values for this soil type (Table 1).

When the plant grew the sixth leaf, the following growth indicators were evaluated: height, dry mass, foliar area, and quality index. Subsequently, the plants were divided by organs (leaves, stems, and root) and were washed with water; they were placed in paper and

Table 1. Chemical properties of the soil and substrate (n=3).

	P_2O_5	K_2O	pH KCl	pH H_2O	M.O.	Ca^{+2}	Mg^{+2}	K^{+}
	mg 100 g^{-1} soil				%	cmol$_c$ kg^{-1}		
Soil	34.21	35.05	6.04	6.97	2.51	48.40	13.14	0.82
Substrate	198.33	134.09	6.17	6.70	5.96	47.67	15.06	2.95

were dried at 70 °C in a forced-air stove, until they reached a constant weight. The plants were then crushed and filtered using a 0.2 mm sieve, before they were subject to a chemical analysis.

The chemical analysis was carried out at the lab of the Estación Territorial de la Caña de Azúcar of Palma Soriano, Santiago de Cuba province. N concentration was determined using the Kjeldahl method; P concentration was determined by colorimetry, using the molydbeno blue method; and K was determined by flame photometry.

Based on the dry matter values and the concentrations, in 2019, nutrient absorption was calculated, while the formula proposed by Siddiqi and Glass (1981) was used to estimate the nutrient use efficiency (EU).

During the statistical processing, the Kolmogorov-Smirnov Goodness of Fit test was used to corroborate the normality of the data and the Levene Test for Equality of the Variances was used for the variance homogeneity. Subsequently, a simple classification of variance analysis was carried out. The differences between treatments were determined using the Duncan's Multiple Range Test ($p\leq 0.05$).

The effect of the product doses on the nutrient absorption and use efficiency was adjusted to several regression models, using the higher coefficient of determination (R^2) as the selection criterion. The maximum values were determined calculating the first derivative from the regression equations.

RESULTS AND DISCUSSION

Seedling growth

The Enerplant® applications had a positive impact on the growth of the cacao seedlings (Table 2).

The treatments were the biostimulant was applied obtained the highest mean values; they statistically surpassed the control (without biostimulant applications) or their results were statistically similar. Overall, a dose of up to 2 mL ha^{-1} of the product significatively increased the growth indicator of the cacao seedlings during both years (Table 2).

The tallest cacao seedlings were obtained with the application of 2 mL ha^{-1} doses of Enerplant®; however, these results were not statistically different from those achieved with the application of 1 mL ha^{-1} doses of the biostimulant, a treatment which significantly

Table 2. Effect of the Enerplant® doses in the growth of cacao seedlings.

Enerplant, mL ha^{-1}	Height (cm)		Total dry mass (g)		Quality index		Leaf area (cm^2)	
	2019	2020	2019	2020	2019	2020	2019	2020
0	27,27 d	32,41 b	2,85 e	4,72 bc	0,267 c	0,503 b	620,73 b	636,34 c
1	34,21 ab	39,59 a	3,93 b	5,74 a	0,559 a	0,541 a	1094,66 a	858,80 a
2	35,25 a	40,45 a	4,45 a	5,17 b	0,379 b	0,465 c	1009,30 a	789,04 ab
3	32,87 bc	38,80 a	3,71 c	4,54 c	0,326 bc	0,330 e	747,09 b	832,77 ab
4	31,33 c	34,61 b	3,20 d	4,56 c	0,274 c	0,415 d	710,03 b	748,15 b
SE, $\bar{x}$	0,56*	0,96*	0,13*	0,19*	0,02*	0,02*	59,51*	31,72*

SE=Standard error. Means with different letters in each column are statistically different (Duncan, $p\leq 0.05$).

increased height in 2019 and 2020 (25% and 22% more than control, respectively) (Table 2). A downwards trend for this indicator was identified when higher doses were applied (3 and 4 mL ha^{-1}). These height values were slightly higher than those reported by Suparno *et al.* (2015) for cacao seedlings evaluated for a longer time (180 days) in Indonesia.

Statistically higher dry mass values were recorded with the 2 mL ha^{-1} (2019) and the 1 mL ha^{-1} (2020) doses (37% and 22% more than other treatments, respectively). Likewise, a downwards trend for the height variable was recorded when higher biostimulant doses were applied (Table 2).

Gonçalvez *et al.* (2018) explain that the ideal balance for the growth of different vegetative organs is variable: a certain endogenous concentration of a given nutrient might favor the growth of one organ and inhibit the growth of another.

During this stage of the cultivation, the leaves, the stem, and the roots account for 59%, 27%, and 14% of the dry mass, respectively (data not shown), when the study doses were applied. These figures are higher than those reported by Osorio *et al.* (2017) who studied various container sizes and established that, 305 days after their emergence, the accumulated biomass of cacao seedlings was mainly distributed among leaves (48%) and the stem (33%), and, to a lesser extent, in the root.

Applying 1 mL ha^{-1} doses of Enerplant® favoured the sucess of cacao seedlings. The highest quality index was obtained during both years with this dose. They were statistically superior to every other of the study treatments (109% and 7% increases during the first and second years, respectively). The 4-mL dose of the biostimulant reduced this indicator during the second year by 17%, compared with the treatment without application (Table 2).

Applying a 1 mL ha^{-1} dose achieved the highest leaf area values for both years (Table 2). These values were statistically superior ($p \leq 0.05$) than the control by 76% and 35%, during the first and second years, respectively. Likewise, an increased dose showed a downwards trends for this variable. The leaf area values were higher than those reported by García (2018) who studied the application of water-soluble fertilizers to seedlings of this crop in Peru.

The positive results of the application of Enerplant® —which are higher than the control treatment corroborate that the product biostimulates vegetative growth, significantly increasing the morphological variables of cacao seedlings. Remarkably, this effect can disappear when doses higher than 1- and 2-mL ha^{-1} are applied.

Nutrient concentration

Compared with control without Enerplant® application, the N and K concentration on the leaves did not increase when the biostimulant was applied (Table 3). Rather, N and K concentrations decreased as the doses increased. However, phosphorous concentration in the three organs analyzed showed a gradual increase and the highest phosphorous values were recorded with the highest biostimulant dose.

The values of N were higher than those reported in Colombia by Puentes-Páramo *et al.* (2016) for the ICS-95, CCN-51, TSH-565, and ICS-39 clones and by De Oliveira *et al.*

Table 3. Effect of Enerplant® doses in the nutrient concentrations of cacao seedling organs (experiment carried out in 2019).

Enerplant, mL ha^{-1}	Leaves			Stem			Root		
	% N	% P	% K	% N	% P	% K	% N	% P	% K
0	22,3 a	2,2 d	25,8 a	16,1 b	4,6 e	21,4 d	18,4 a	3,0 e	15,9 d
1	22,6 a	2,2 d	25,4 a	16,0 c	5,0 d	26,1 b	17,0 b	3,1 d	18,3 c
2	20,9 b	2,4 b	25,4 a	16,8 a	5,5 c	26,5 a	15,7 c	3,6 b	18,8 b
3	20,4 c	2,3 c	23,8 b	15,4 d	5,6 b	26,5 a	16,1 c	3,3 c	19,2 a
4	19,9 d	2,5 a	24,0 b	16,1 b	5,9 a	25,1 c	14,3 d	3,7 a	19,0 b
SE, $\bar{x}$	0,13*	0,019*	0,15¨*	0,01	0,001	0,01	0,14	0,028	0,07

SE=Standard Error. Means with different letters in each column are statistically different (Duncan, p≤0.05).

(2019) for the CCN 51 and PS1319 clones in Brazil. The P and K concentrations in this experiment were likewise higher than the concentrations reported by the said authors. The concentrations fell within the adequate range for N (22-25 g kg^{-1}) and P (1.5-2.5 g kg^{-1}) concentrations established for high-productivity cultivation areas in Brazil (Marrocos *et al.*, 2020). The high nutrient values determined in this experiment could be related to the substrate fertility, as well as to the age of the material used (4 months), while the values reported by Puentes-Páramo *et al.* (2016) belong to established plantations and have therefore greater dry mass, which might have been the result of the lixiviation effect.

Nutrient absorption and use efficiency

The nutrient absorption by cacao during the nursery stage showed a K>N>P pattern, regardless of the organ that was analyzed and the Enerplant® doses that was applied (Table 4).

Increasing the Enerplant® doses (up to 2 mL ha^{-1}) increased nutrient absorption. Greater doses resulted in a diminishing trend of absorption values (Table 4) —except for K values in the root.

In their study about the use efficiency of N, Ribeiro *et al.* (2008) did not find differences between the study's cacao clones, although they did notice that the efficiency of that indicator increased along with the nitrogen doses.

Table 4. Effect of the Energplant® doses in nutrient absorption by cacao seedings (mg $organ^{-1}$), during the 2019 experiment.

Enerplant, mL ha^{-1}	Leaves			Stem			Root		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
0	36,22 d	4,40 e	95,95 c	12,71 c	4,38 e	38,70 d	7,82 c	1,53 c	15,64 b
1	56,59 a	6,89 b	145,68 a	12,60 c	4,74 d	47,08 c	10,78 a	2,37 ab	26,60 a
2	53,06 b	7,62 a	147,69 a	21,60 a	8,52 a	78,07 a	9,88 ab	2,71 a	27,06 a
3	46,18 c	6,51 c	123,43 b	13,98 b	6,13 c	55,13 b	8,61 bc	2,13 b	23,55 a
4	36,32 d	5,69 d	100,32 c	14,65 b	6,47 b	52,33 b	7,60 c	2,37 ab	23,14 a
SE, $\bar{x}$	0,71*	0,09*	1,82*	0,29*	0,12/*	1,06*	0,53*	0,13*	1,35*

SE=Standard error. Means with different letters in each column are statistically different (Duncan, p≤0.05).

The nitrogen absorption values recorded in this study for the above-ground areas (leaves+stems) were similar to the findings of Ribeiro *et al.* (2008) for seven-month-old cacao organs fertilized with a doses equivalent to 120 mg of N per 6-kg pot; however, the estimated nitrogen absorption values recorded for the root were lower than the results of the same authors.

Cabala-Rosand and Mariano (1985) discovered a close relationship between the phosphorous content in the roots of cacao seedlings and the size of the roots; they also found that longer cultivars with greater root area absorbed more environmental P. However, Ribeiro *et al.* (2008) believed that, in the case of perennial cacao crops, nutrient concentration in the roots may not be a proper indicator for the selection of efficient genotypes, at least in the case of young plants, whose nutrient concentration is also influenced by their growth conditions and size.

Bulgari *et al.* (2019) and Yakhin *et al.* (2017) explain that the complex nature of the composition of biostimulants and the wide range of molecules that they include make it difficult to understand and define which are the most active compounds. The action of biostimulants is a consequence of the synergic action of the various bioactive molecules.

Regardless of the Enerplant® dose applied, the nutrient use efficiency was higher for P, followed by N and finally by K (Table 5). The highest nitrogen and potassium use efficiency values were higher when a 2 mL ha^{-1} dose was applied, while the highest P values were obtained with the 1 mL ha^{-1} dose.

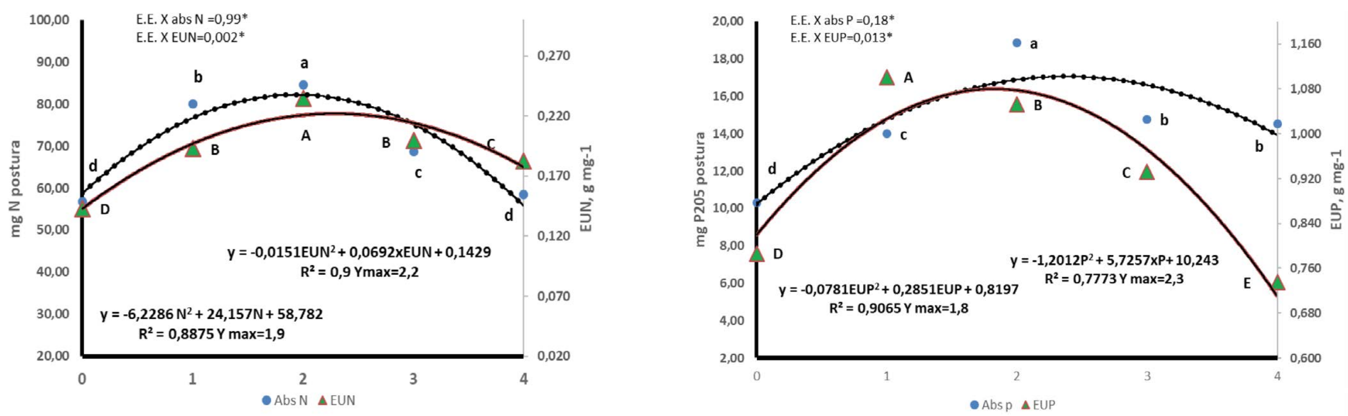
Correlating the biostimulant doses with the absorption values and the use efficiency showed the highest adjustment to the quadratic functions (Figure 1). Applying a 2 mL dose of Enerplant® per hectare resulted in the highest nutrient absorption and use efficiency values by cacao seedlings, which were statistically different from the values recorded when the rest of the doses were applied. Applying higher biostimulant doses resulted in a diminishing trend for both variables.

Ferreira *et al.* (2017) studied the effect of five concentrations of a biostimulant made of algae extract in the vegetative growth of grape seedlings and determined that increasing the dose reduced the evaluated variables. The increase in the high doses of biostimulants diminished metabolic processes (Ferreira *et al.*, 2018).

Table 5. Nitrogen, phosphorous, and potassium efficiency use by cacao seedlings in 2018 (g dry mass mg $nutrient^{-1}$).

Enerplant®, mL ha^{-1}	**NUE**	**PUE**	**KUE**
0	0,143 d	0,787 d	0,054 d
1	0,193 b	1,101 a	0,070 b
2	0,235 a	1,052 b	0,078 a
3	0,200 b	0,931 c	0,068 b
4	0,182 c	0,735 e	0,061 c
SE, $\bar{x}'$	0,002*	0,013*	0,0009*

SE=Standard Error. *Means with the same letters do not differ for $p \leq 0.05$ according to Duncan's test. NUE=nitrogen utilization efficiency; PUE=phosphorus utilization efficiency and KUE=potassium utilization efficiency).



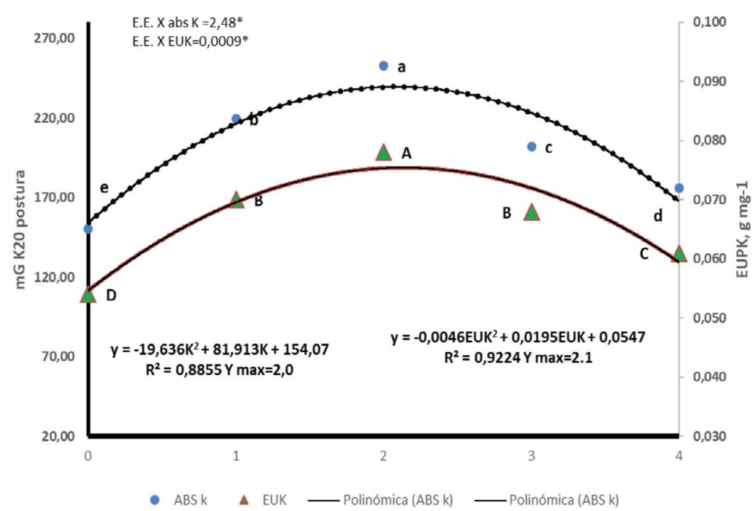


Figure 1. Relationship between Enerplant® doses and nutrient absorption and use efficiency by cacao seedlings, during the 2018 experiment. Means with different letters in each column are statistically different (Duncan, p≤0.05). Upper case letters indicate different mean use efficiency values. Lower case letters indicate nutrient absorption values. Legend in capital letters the significance for the differences in the mean values of utilization efficiency. In lower case for nutrient absorption values. From left to right and top to bottom: nitrogen, phosphorus and potassium.

CONCLUSIONS

Theobroma cacao L. seedlings had a positive response to the application of Enerplant®. Likewise, it significantly increased the morphological variables of cacao seedlings. In average, during the two years of the study, a 1 mL ha^{-1} dose of Enerplant® significantly increased the height (24%), dry mass (30%), quality index (58%), and leaf area (56%) of the cacao seedlings. Doses above 1- and 2- mL ha^{-1} diminished seedling growth. Finally, applying a 2 mL ha^{-1} dose of biostimulant guaranteed greater N, P, and K absorption and use efficiency values by cacao seedlings.

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AP AGRO PRODUCTIVIDAD

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Exploratory study: testicular microlithiasis in cattle from the Costa Chica region

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ABSTRACT

Objective. To determine the presence of microlithiasis in bulls from the Costa Chica, Guerrero, Mexico, and to assess the sperm quality of the bulls affected by this pathology.

Methodology. Seventy-seven bulls were assessed in four municipalities of the state of Guerrero, Mexico. Bull testicles were subjected to an ultrasonography test and, in the event of microlithiasis, they were classified according to the number of points observed. In addition, semen was collected by electroejaculation. Sperm concentration ($\times 10^6$ sperm mL^{-1}) and individual motility (%) were quantified from the samples. Microlithiasis results are expressed as a percentage of the total number of assessed animals. Seminal characteristics were subjected to an ANOVA and the means were compared using the Tukey test.

Results. Approximately 25% of the assessed individuals showed microlithiasis. About 60% of such cases were classified as grade 2 (>5, <25 points). Three microlithiasis grades were identified in Brown Swiss bulls, while in Gyr bulls no grade was identified. Regarding seminal characteristics, in average, the overall sperm concentration was 992.5×10^6 sperm mL^{-1} and the overall individual motility was 75.5%. The bull breed variable did not generate statistical differences in the seminal quality variables.

Conclusion. The presence of microlithiasis was identified in bulls from the Costa Chica region in Guerrero; however, sperm concentration and individual motility were not affected.

Key terms: Testicular diseases, ultrasound scan, semen.

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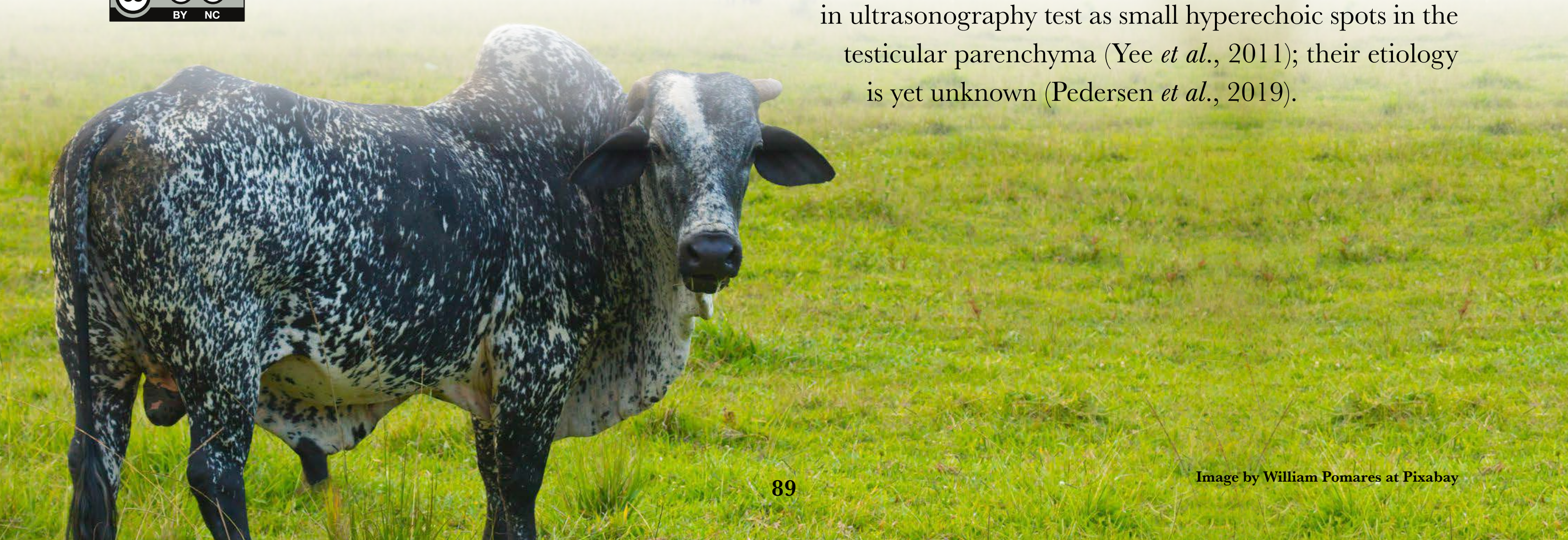
INTRODUCTION

During the selection of the breeding male, particular attention should be paid to the ejaculated semen quality (Kabiraj *et al.*, 2011) which determines the fertilizing capacity (Großfeld *et al.*, 2008). The conditions that can affect bull fertility include pathologies such as testicular microlithiasis (Chandolia *et al.*, 2018). This condition is defined as calcifications found in the seminiferous tubules (Tan and Eng, 2011). It can be observed in ultrasonography test as small hyperechoic spots in the testicular parenchyma (Yee *et al.*, 2011); their etiology is yet unknown (Pedersen *et al.*, 2019).



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In case of animals of zootechnical interest, this pathology can be considered an incidental finding when bulls are assessed (Chandolia *et al.*, 2018). One of the concerns arising from this pathology is its potential effect on spermatogenesis (Sihag *et al.*, 2018), sperm quality, and testosterone concentration. Recent studies in humans have found an inversely proportional relationship between microlith presence and sperm quality (Xu *et al.*, 2014).

In Mexico, cattle raising is the second most widespread productive activity in rural areas (Rodríguez Mejía *et al.*, 2018). However, meat and milk production levels are not optimal. A similar situation is found in the Costa Chica region of the state of Guerrero, where livestock is developed under tropical conditions, free grazing prevails, and reproductive programs are based on natural mating. To improve productive and reproductive indexes, the bulls with the best semen characteristics should be identified, avoiding breeders with low fertility or testicular tissue diseases. Therefore, the objective of this study was to determine whether or not microlithiasis is found in bulls from the Costa Chica in Guerrero, Mexico and to assess the sperm quality of males affected with such pathology.

MATERIALS AND METHODS

Location

The study was performed in four municipalities of the Costa Chica region, in the state of Guerrero, Mexico (Azoyú, Cuajinicuilapa, Ometepec, and San Marcos). The region has a predominately warm sub-humid climate with rains in summer, and temperature fluctuates between 22 and 28 °C. Precipitation ranges from 1200 to 2000 mm.

Assessed animals

From July 2018 to July 2019, seventy-seven bulls (*Bos indicus*, *Bos taurus*, and *Bos indicus* × *Bos taurus*) from six different breeds were assessed (Table 1). The following inclusion criteria were used: age ≥16 months; and body condition ≥4, on a scale from 1 (emaciated) to 9 (obese) (Ayala-Burgos *et al.*, 1998). The scrotal circumference was measured using a scrotimeter, in order to verify that it was appropriate for their race and age (Momont and Checura, 2015). A full clinical evaluation was performed to rule out the presence of reproductive pathologies that could affect the testicles or sperm quality.

Table 1. Analyzed bulls grouped by breed and place of origin.

Breed	Municipality			
	Azoyú	Cuajinicuilpa	Ometepec	San Marcos
Brahman	4	6	3	8
Gyr	-	4	1	3
Gyr-Holando	-	-	-	2
Sardo Negro	-	7	-	2
Suiz-Bú	1	4	-	5
Brown swiss	3	5	11	8

In general, bulls are kept under grazing conditions, with mineral salt and water supplied *ad libitum*. As health measures, farmers apply vaccines against rabies, tuberculosis, and other diseases, according to the official vaccination plans established by the regional health authorities.

Testicles ultrasound examination

Testicular parenchyma was evaluated in the dorsoventral and mediolateral direction as indicated by the Momont and Checura methodology (2015), using an ultrasonograph (Chison Eco 5, China with a 7.5-MHz linear transducer). In the event of testicular microlithiasis, it was classified using a scale from 1 to 3, where 1 represents <5 hyperechoic points, 2 represents 5-25 hyperechoic points, and 3 represents >25 hyperechoic points (Amarawardena and Siyambalapitiya, 2016).

Semen collection and assessment

Semen was gathered using an electroejaculation equipment (Standard Precision, USA), collecting only the portion rich in spermatozoa. Sperm concentration was estimated using a Neubawer chamber, with the technique described by Dearing *et al.* (2014). Individual motility (IM) was evaluated by placing a semen drop under a cover slip, using a 40X objective and observing several fields. IM was quantified on a 1-100% scale (a uniform rectilinear motion), according to the Páez-Barón and Corredor-Camargo (2014) technique.

Statistical analysis

The microlithiasis incidence is showed as a percentage of the total number of animals involved in the study.

Semen quality data was subject to an analysis of variance using an unbalanced completely randomized design. The independent variable was the bull breed (Brahman, Gyr, Girolando, Sardo Negro, Suiz-Bu, and Brown Swiss), and the response variables were sperm concentration and individual motility. Treatment means (bull breed) were compared with Tukey's test (α=0.05). All statistical analyzes were performed with the SAS software.

Ethics standards compliance

No animal testing was involved in this work, and it was performed in accordance with procedures accepted by the Universidad Autónoma de Guerrero.

RESULTS AND DISCUSSIONS

Table 2 shows the percentage of individuals in which microlithiasis was identified, as well as the microlithiasis degree, based on the number of hyperechoic points observed (Figure 1a-c). Out of the total assessed animals, 24.7% showed some microlithiasis degree. More than 50% of the total number of animals in which microlithiasis was identified belong to degree 2 (Figure 1b). The most affected breed was the Brown Swiss breed (57.9% of the observed cases), followed by the Brahman breed (26.3%). Likewise, the Brown Swiss breed was the only one in which the 3 microlithiasis degrees were observed (Figure 1c).

Table 2. Microlithiasis percentage in bulls from different breeds used in the Costa Chica, Guerrero, Mexico.

Breed	N	Age (months) Mean ± SD	Microlithiasis[z]			
			NI	1	2	3
Brahman	21	37.1±17.12	20.8	5.2	1.3	-
Gyr	8	41.8±20.19	10.4	-	-	-
Gyr-Holando	2	48.0±0.00	1.3	-	1.3	-
Sardo Negro	9	29.8±7.79	11.7	-	-	-
Suiz-bú	10	38.0±23.32	10.4	1.3	1.3	-
Brown Swiss	27	39.1±24.48	20.8	2.6	10.4	1.3

[z] Microlithiasis degree: Degree 1, <5 hyperechoic points; Degree 2, 5-25 hyperechoic points; and Degree 3, >25 hyperechoic points, NI: Not identified.

Table 3 shows the means of sperm concentration ($\times 10^6$ sperm mL^{-1}) and individual motility (IM, %) observed in cattle of different breeds in the Costa Chica. The overall average for sperm concentration was $992.5 \pm 782.5 \times 10^6$ sperm mL^{-1}, with a range between 25.0 and 2375×10^6 sperm mL^{-1}. Moreover, the individual motility percentage had an average of 75.5±23.54%, with a range between 10 and 98%. Gyr and Brahman bulls showed the highest magnitude values for both characteristics. The bull breed variable did not generate a statistical difference with regard to sperm concentration ($p=0.4531$) or individual motility ($p=0.4531$).

The microlithiasis presence in the testicular tissue is uncommon and even rare (Chandolia *et al.*, 2018); it is usually reported as an incidental finding. In human males, Furness *et al.* (1998) and Catanzariti *et al.* (2014) have reported between 0.6% and 12.7% microlithiasis presence, respectively; those percentages are lower than those obtained in this study. In case of animals of zootechnical interest, this pathology has been identified in goats, rabbits, horses (McEntee, 1990), and buffalo (Chandolia *et al.*, 2018); this pathology

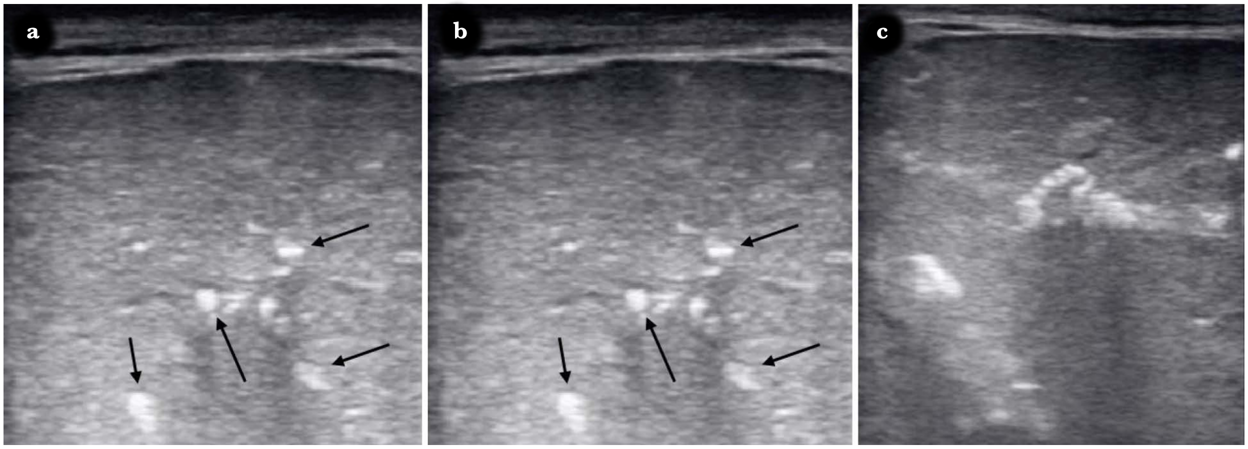

Figure 1. Testicular microlithiasis degree according to the number of observed hyperechoic points. a. Degree 1, <5 hyperechoic points. b. Degree 2, 5-25 hyperechoic points. c. Degree 3, >25 hyperechoic points. In a and b, the arrows indicate the microliters.

Table 3. Sperm concentration and individual motility in bulls from different breeds used in the Costa Chica, Guerrero, México.

Breed	n	Age (months)	Sperm concentration (1×10^6 sperm mL^{-1})	Individual motility (%)
Brahman	7	38.7±11.8	1150.0±791.7 a	84.7±7.3 a
Gyr	2	50.5±30.4	1875.0±707.1 a	87.5±3.5 a
Sardo Negro	4	24.8±9.4	756.3±821.9 a	46.7±5.8 a
Suiz-Bú	6	36.8±31.4	808.3±827.9 a	70.0±30.8 a
Brown swiss	11	34.0±14.7	918.2±763.2 a	73.0±26.2 a

Means ± SD with the same letters indicate that there is no statistical difference (Tukey, 0.05).

was recorded in 33% of the analyzed cases of the latter species —a higher value than that obtained in this research. However, no studies showing the presence of this pathology in cattle were identified, because the testicular tissue examination is generally performed by manual palpation, a low sensitivity procedure that does not provide a comprehensive diagnosis.

In this study, the sperm concentration obtained was higher than 700×10^6 sperm mL^{-1} and, in most cases, individual motility was higher than 50%. These values are considered normal and are higher than the ranges reported by Páez-Barón and Corredor-Camargo (2014) and Vélez-Castañeda *et al.* (2014). The differences may be related to the age range and the bull management in the various studies. The number of individuals analyzed does not allow to establish a final conclusion regarding whether or not the breed creates a statistical difference on the analyzed semen characteristics; however, evidence suggests that breed is not a variation source for the said characteristics (Orantes *et al.*, 2010; Vélez-Castañeda *et al.*, 2014).

CONCLUSIONS

The results obtained in this research show the presence of microlithiasis in bulls from the Costa Chica region, Guerrero, Mexico. However, no decrease in the mean values of sperm concentration or individual motility was observed. More studies are necessary —with a larger size of the studied population as well as more seminal variables—, in order to identify factors that favor microlithiasis development in that region and to study the relationship of this pathology with other seminal quality and fertility parameters.

CONFLICT OF INTEREST

The authors declare and agree with the information submitted in this article and accept the order of appearance in the document. There is no conflict of interest to declare.

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AP AGRO PRODUCTIVIDAD

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Performance of soybean cultivars under drought stress and sowing seasons in Brazilian Savannah

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ABSTRACT

Objective: Evaluate the performance of two soybean cultivars submitted to water deficit in two sowing seasons (July 10, 2019, and October 27, 2020), in Tocantinense Savannah, Brazil.

Methodology: The experiments were carried out in a greenhouse, in pots. The experimental design used in each experiment was completely randomized in a 4×2 factorial scheme with four replications, represented by four irrigation management systems (water deficit in the flowering, grain filling and maturation stages, and without water deficit) and two cultivars (TMG132RR™ and TMG1288RR™). The means were grouped by the Scott-Knott test at 5% significance. The characteristics evaluated were: number of seeds per plant, mass of one hundred seeds in grams, number of pods per plant, plant height and grain yield per hectare (GY) in kilograms.

Results: In the two seasons, the water deficit during grain filling affected the number of pods and seeds per plant, the 100 seeds mass, and the grain yield of both cultivars.

Implications: The water availability and sowing seasons are environmental factors with the greatest impact on cropping. Therefore, understanding how cultivars behave in adverse environmental situations is of great importance to management programs.

Conclusions: The cultivar TMG132RR™ was less sensitive to water deficit, whereas the cultivar TMG1288RR™ was more productive under adequate conditions of temperature and water availability.

Keywords: Available water; environmental stress; [*Glycine max* (L.) Merrill]; irrigation.

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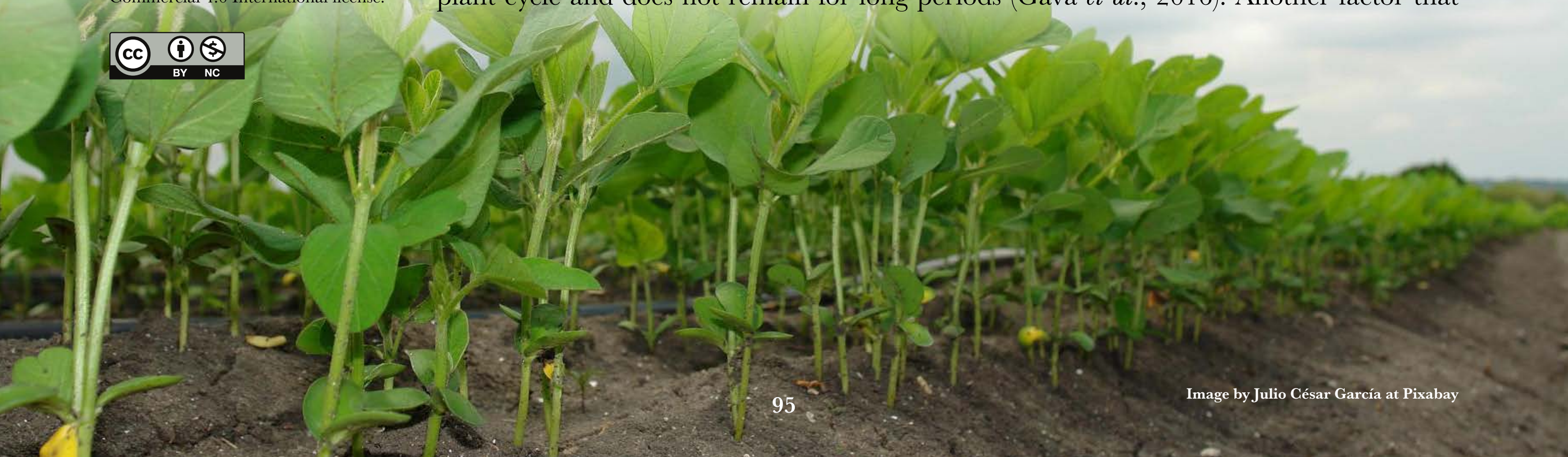
INTRODUCTION

Water availability and sowing seasons are environmental factors considered to have a major impact on crop yield. Soybeans [*Glycine max* (L.) Merrill] are considered to be tolerant to water deficit, as long as this condition does not occur in critical stages of the plant cycle and does not remain for long periods (Gava *et al.*, 2016). Another factor that



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adds up to the effects of drought is temperature, since plants subjected to the same water depth but at different temperatures generally present different physiological responses.

At high temperatures, Pípolo (2002) observed changes in nitrogen availability for grains in soybean plants and, consequently, variations in protein content, producing seeds with low commercial value.

When the water deficit occurs during flowering and beginning of pod formation, the effects are reflected on the abortion of flowers, and, later, on the size and chemical composition of the grains (Mundstock; Thomas, 2005).

Another important aspect related to the response to drought is the crop plasticity. Soybean has high plasticity, *i.e.* the ability to adapt to environmental and management conditions through changes in its morphology and yield components (Komatsu *et al.*, 2010). However, it is important to consider that different cultivar can respond differently to these environmental variations.

For this reason, understanding how cultivars behave in adverse environmental situations is of great importance to management programs, aiming to minimize losses in unusual situations.

Thus, the present study aimed to evaluate the performance of two soybean cultivars, submitted to water deficit, in different reproductive stages and sowing seasons, in the state of Tocantins, Brazil.

MATERIAL AND METHODS

Location and Characterization of Experimental Area

Two experiments were carried out in a greenhouse, on the campus of the Federal University of Tocantins, municipality of Palmas, Brazil the first being installed on July 10, 2019, and the second on October 27, 2019.

According to the Köppen climate classification, the climate in this region is a humid tropical type with a well-defined dry season (Aw) in winter. The average annual potential evapotranspiration is 1500 mm, with average annual temperature and precipitation of 27.5 °C and 1600 mm, respectively (INMET, 2015). The climatic data were collected from a mobile experimental station installed near the greenhouse (Figure 1).

The soil in the experimental area is red yellow dystrophic oxisol with textural class loam sandy (sand-82%; clay-13%; silt-5%), pH-4.9; organic matter 6 $g.dm^{-3}$. The average value of soil density was 1.55 $g\ cm^{-3}$. Fertilization was carried out based on the recommendation for soybean cropping (EMBRAPA, 1999).

Conduction of Experiments

The experimental design in each experiment was completely randomized with four repetitions for treatment. The treatments were arranged in a 4×2 factorial scheme, represented by four irrigation systems (WDF> water deficit in flowering R1-R3, WDG> water deficit in grain filling R4-R5.5, WDM> water deficit in maturation R6-R7, and NI> normal irrigation/control) and two cultivars (TMG132RR™ and TMG1288RR™, both of medium cycle and determined growth habit).

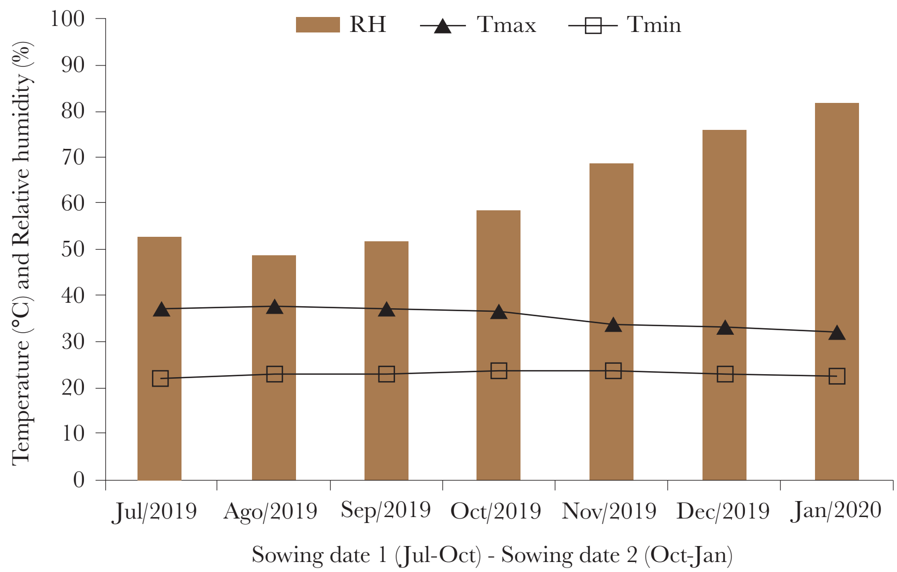

Figure 1. Climatic data (maximum, minimum, and relative humidity) of the experimental area at the Federal University of Tocantins, in the municipality of Palmas, Brazil, obtained during the experiment conduction (from July 2019 to January 2020).

The experimental plot was represented by polyethylene pots, with a volumetric capacity of eight liters. The seeds were sown manually. After emergence, the plants were thinned to leave two plants per pot.

The water balance was carried out through the physical–hydraulic characteristics of the soil (field capacity – FC and permanent wilting point - PWP), determined *in situ*. The available soil water was calculated by the difference between field capacity and permanent wilt point. Irrigation management was performed with an adaptation of the methodology proposed by Sinclair and Ludlow (1986). For WDF, WDG and WDM, a variation of up to 30% of the water available in the soil was maintained. For NI, the volume always remained close to 70% of the water available in the soil.

Evaluated characteristics

The agronomic characteristics evaluated were: number of seeds per plant (NSP), mass of one hundred seeds (M100) in grams, number of pods per plant (NPP), plant height (PH) in centimeters, and grain yield per hectare (GY) in kilograms.

Statistical Analysis

The treatments were subjected to individual analysis of variance, and then to joint analysis (Cruz; Regazzi, 2004). The means were grouped by the Scott-Knott (1974) test at 5% significance. The statistical program SISVAR version (5.0) was used.

RESULTS AND DISCUSSION

The summary of the joint analysis of variance for the characteristics number of seeds per plant (NSP), mass of one hundred seeds (M100) in grams, number of pods per plant

(NPP), plant height (PH) in centimeters, and grain yield in kilograms per hectare (GY) is shown in Table 1.

For all characteristics, significant effects were detected for IM and S, demonstrating the importance of water supply management and planting period.

There was a significant difference for all characteristics for the interaction S×IM. As for the interaction S×C, a significant effect was only detected in two characteristics, revealing the similar behavior of cultivars in the two analyzed periods, and differing from the other characteristics.

The interaction IM×C resulted in a significant effect on the NSP, NPP, and GY. As for the triple interaction S×IM×C, only NSP showed no statistical difference between treatments. In this case, this interaction reflects the differential behavior of the cultivars according to the sowing seasons and irrigation management.

Comparison of means

In the S×MI interaction (Table 2), the number of seeds per plant NSP, for all irrigation managements, was higher in the second season (S2) compared to the first season (S1), probably due to the occurrence of milder temperatures recorded throughout the cycle in S2 (Figure 1).

Among the irrigation managements, in the two studied sowing seasons, the highest number of NSP occurred during maturation (WDM) and normal irrigation (NI). At maturation, the occurrence of water deficit did not result in losses in NSP, as at this stage the plants were at the beginning of physiological maturation and the pods were already full, that is with the grains already occupying the entire cavity of the pods. Conversely, lower NSP values were observed in flowering (WDF) and grain filling (WDG), as a result of the harmful effect of water deficit in these stages, corroborating the results reported by Palharini (2016).

Table 1. Analysis of variance related to the characteristics: number of seeds per plant (NSP), mass of one hundred seeds (M100), number of pods per plant (NPP), plant height (PH) and grain yield (GY) of two soybean cultivars.

Source of Variation	GL	NSP	M100 (g)	NPP	PH (cm)	GY (kg)
RE(S)	6	37.01^{ns}	0.96^{ns}	8.86^{ns}	12.83^{ns}	32518.72^{ns}
S	1	27340.62**	93.05*	561.09*	5003.79*	34063169.54*
IM	3	5910.94**	35.27*	576.41*	90.00*	6849649.17*
C	1	252.81^{ns}	32.56*	718.91*	546.97*	306824.45^{ns}
S×IM	3	2708.54**	12.88*	191.38*	41.67*	2786878.26*
S×C	1	556.96*	5.92^{ns}	659.84*	52.38^{ns}	25816.49^{ns}
IM×C	3	523.50**	1.27^{ns}	314.26*	36.99^{ns}	818495.60*
S×IM×C	3	305.12^{ns}	5.54*	172.68*	50.60*	1110791.62*
Error	42	128.85	1.68	18.13	15.54	164751.97
CV%		11.96	9.93	13.77	9.23	12.82

Note: * Significant at 5% probability by the F–test; ns–Not significant. RE: repetition; S: sowing seasons; IM: irrigation management; C: cultivar; CV%: coefficient of variation.

Table 2. Analysis of the interaction S×IM and of the interaction IM×C for the number of seeds per plant (NSP), in Palmas, Tocantins, Brazil.

IM	Number of seeds per plant (NSP)			
	Sowing Seasons		Cultivars	
	S1	S2	TMG 132RR™	TMG 1288RR™
WDF	33.21bB	49.62aA	50.77cA	32.06bB
WDG	20.43bB	41.93bA	33.12dA	29.25bA
WDM	39.37aB	97.81aA	60.00bA	69.18aA
NI	41.62aB	102.62aA	73.37aA	70.87aA

Means followed by the same lowercase letter, in each column, and uppercase (between the two sowing season; between the two cultivars), in the row, belong to the same statistical group, at 5% significance by the Scott–Knott test. * WDF: water deficit in the flowering stage; WDG: water deficit in the grain–filling stage; WDM: water deficit in the maturation stage; NI: normal irrigation (no water deficit); S1: first sowing season (July 10, 2019); S2: second sowing season (October 27, 2019).

Lima *et al.* (2009) argue that in adverse conditions such as water restriction, the plant will preferably form few seeds in the fixed pods since the biological objective is to guarantee reproduction. The water deficit during flowering (WDF) reduced the number of seeds per plant in cultivar TMG1288RR™ (32.06) compared to cultivar TMG132RR™ (50.77) (Table 2).

Normally, water deficit during flowering causes flower abortion and prevents anthesis (Casagrande, 2001), due to the decrease in photo-assimilated compound translocation from leaves to flowers (Kramer; Boyer, 1995), making seed formation unfeasible. This observed difference may indicate different response mechanisms to water deficit between the cultivars evaluated in this study.

According to Beever (2000), drought tolerance is a polygenic characteristic that is difficult to identify. A specific physiological response to water deficit represents combinations of molecular events that are triggered by the perception of stress (Bray, 1993). Understanding how these events interact with each other is important for identifying drought-tolerant cultivars and enabling breeding programs for this purpose.

The results of the triple S×IM×C interaction for plant height (PH) and number of pods per plant (NPP) characteristics are shown in Table 3.

For plant height (PH), there were no differences between the irrigation management, in the two evaluated sowing seasons, for TMG132RR™, showing stability for this characteristic, even in unfavorable environmental conditions. Conversely, for the cultivar TMG1288RR™, irrigation management effects were observed in each of the sowing seasons. The PH reduction observed for TMG1288RR™ submitted to water deficit in S1 may have occurred in response to the environmental stress imposed during the grain-filling (Figure 1). As for S2, also for TMG1288RR™, possibly the genetic factor was decisive, since there was no water deficit in NI, and, during WDM, plants had already reached the maximum height.

The sowing season did not influence the NPP of the cultivar TMG132RR™ (Table 3). Conversely, for TMG1288RR™, was observed greater NPP in S2 during WDM and

Table 3. Analysis of the S×IM×C, for the plant height (PH) and number of pods per plant (NPP), in Palmas, Tocantins, Brazil.

MI	Plant height (cm)				Number of pods per plant (NPP)			
	TMG132RR™		TMG1288RR™		TMG132RR™		TMG1288RR™	
	S1	S2	S1	S2	S1	S2	S1	S2
WDF	48.97aA1	34.00aB1	48.62bA1	37.37aB1	19.00aA1	17.37aA1	13.37bA1	14.25bA1
WDG	45.25aA2	28.50aB1	54.12bA1	31.62bB1	8.12bA1	8.12bA1	12.37bA1	12.87bA1
WDM	51.00aA1	27.50aB2	52.87bA1	40.87aB1	13.37bA1	13.00bA2	17.37aB1	42.00aA1
NI	52.87aA1	30.12aB2	58.62aA1	40.92aB1	18.75aA1	18.75aA2	17.39aB1	40.62aA1

1=Comparison between irrigation managements: means followed by the same lowercase letter in the column, for the same sowing seasons and cultivar, belong to the same statistical group, at 5% significance by the Scott–Knott test. 2=Comparison between sowing seasons: means followed by the same upper case letter in the row, for the same cultivar and irrigation management, belong to the same statistical group, at 5% significance by the Scott–Knott test. 3=Comparison between cultivars: means followed by the same number, for the same sowing season and irrigation management, belong to the same statistical group, at 5% significance by the Scott-Knott test. * WDF: water deficit in the flowering stage; WDG: water deficit in the grain-filling stage; WDM: water deficit in the maturation stage; NI: normal irrigation (no water deficit); S1: first sowing season (July 10, 2019); S2: second sowing season (October 27, 2019).

NI, since at this time the temperature and humidity conditions were more favorable (Figure 1). This indicates that under more favorable environmental conditions, the cultivar TMG1288RR™ had a better response to NPP.

Comparing the irrigation managements within each cultivar, the cultivar TMG132RR™ showed a reduction of NPP during WDG and WDM. For TMG1288RR™, the lowest NPP values occurred in WDF and WDG (Table 3).

According to Gava *et al.* (2015), the reproductive phase is more sensitive to water stress, which reinforces the results found in the study for this characteristic.

As for the cultivars within each season and irrigation management, the cultivar TMG1288RR™ showed higher NPP in S2 for WDM and NI (Table 3).

This response can often be related to the crop plasticity, which can vary among cultivars (Ludwing *et al.*, 2011), a fact that may have resulted in differences between the cultivars TMG132RR™ and TMG1288RR™. It is important to note that although the largest size of cultivar TMG1288RR™ occurred in S1, compared to S2, the largest number of pods per plant (NPP) occurred in S2 in comparison to S1, for the WDM and NI management systems.

Normally, larger plants have greater water demand, compromising the formation of pods and the grain-filling (Farias *et al.*, 2007). In a study conducted by Balbinott Júnior *et al.* (2018), the authors observed that the number of pods per plant was the variable that most contributed to explaining the phenotypic plasticity in soybean plants.

As for the mass of 100 seeds (M100) (Table 4), for the cultivar TMG132RR™, the water deficit in S1 provided a reduction in all treatments, differing only from normal irrigation. In S2, only WDF differed from the others, resulting in a higher average. For TMG1288RR™, the management that promoted the lowest M100 values was WDM, in S1, and WDM and WDG, in S2.

The comparative study between sowing seasons, in each cultivar and irrigation management system, revealed a reduction in M100 only in WDF, for cultivar TMG132RR™ and in WDF, WDM, and NI for cultivar TMG1288RR™ (Table 4).

Table 4. Analysis of the interaction S×IM×C, for the characteristic mass of 100 seeds (M100) and grain yield (GY), in Palmas, Tocantins, Brazil.

IM	Mass of 100 seeds (g)				Grain yield (kg)			
	TMG132RR™		TMG1288RR™		TMG132RR™		TMG1288RR™	
	S1	S2	S1	S2	S1	S2	S1	S2
WDF	6.38bB1	11.10aA1	8.64aB1	12.82aA1	691.46bB1	2240.19bA1	801.38bB1	1292.79bA2
WDG	6.33bA1	7.32bA1	7.69aA1	7.59cA1	316.78cB1	1097.26cA1	564.94cB1	785.29cA2
WDM	6.21bA1	7.87bA1	5.68bB1	10.57bA1	544.17bB1	2168.72bA2	554.46cB1	3301.84aA1
NI	9.33aA1	9.18bA2	9.62aB1	12.63aA1	1168.98aB1	2890.92aA2	1193.86aB1	3731.76aA1

1=Comparison between irrigation managements: means followed by the same lowercase letter in the column, for the same sowing season and cultivar, belong to the same statistical group, at 5% significance by the Scott–Knott test. 2=Comparison between sowing seasons: means followed by the same upper case letter in the row, for the same cultivar and irrigation management, belong to the same statistical group, at 5% significance by the Scott-Knott test. 3=Comparison between cultivars: means followed by the same number, for the same sowing season and irrigation management, belong to the same statistical group, at 5% significance by the Scott-Knott test. * WDF: water deficit in the flowering stage; WDG: water deficit in the grain–filling stage; WDM: water deficit in the maturation stage; NI: normal irrigation (no water deficit); S1: first sowing season (July 10, 2019); S2: second sowing season (October 27, 2019).

For grain yield, in both cultivars, and in S1 and S2, the management that most compromised yield (GY) was the water deficit during grain-filling (WDG) (Table 4), which also reduced NSP (Table 2), NPP (Table 3), and M100 (Table 4).

The drought stress during grain filling also affected the mass of 100 seeds (M100). According to Albrecht (2008), water restriction can accelerate maturation and reduce the period of reserve accumulation, resulting in grains of stressed plants that not show the normal pattern of development.

Grain yield is a direct function of components such as NPP, PH, NSP, and M100, and depends on a balance between photosynthesis and translocation of its products. Under environmental stress conditions, the plant tries to compensate for cellular damage by adjusting metabolic pathways to ensure reproduction. These mechanisms may vary according to the genotype, stress intensity, and phase in which they occur.

For GY, among the cultivars, no differences were detected in the first sowing season. In the second sowing season, there were different responses of the cultivars in relation to the irrigation managements, as the cultivar TMG132RR™ presented higher grain yield mean in WDF and WDG, while the cultivar TMG1288RR™ was more productive in WDM and WDG.

Considering that the reproductive phase presents more water demand and is most sensitive to water variations, mainly in flowering and grain–filling, it can be inferred that the cultivar TMG132RR™ was less sensitive to drought stress. In contrast, under more favorable conditions, with wide water availability (NI) and milder temperatures (S2), the cultivar TMG1288RR™ was the most productive.

CONCLUSION

The drought stress influenced the agronomic characteristics, cultivars, and sowing seasons. The cultivar TMG132RR™ was less sensitive to drought stress. Under favorable conditions of temperature and water availability, the cultivar TMG1288RR™ was more productive.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Grapevine viruses in Mexico: studies and reports

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ABSTRACT

Objective: To contribute to the knowledge of the diversity of viruses and the viral diseases reported in grapevines in Mexico, in order to benefit producers and develop comprehensive viral disease control strategies.

Design/methodology/approach: The literature search was conducted in databases such as Scopus, Google Scholar, and EBSCO host, using the following keywords alone or in combination: "virus", "plant", "grapevine", and "Mexico". In addition, the INIFAP database was consulted, alongside undergraduate and postgraduate dissertation theses.

Results: Only one academic file was found published in an indexed international journal, using the publication finder software; the report corresponds to a grapevine virus present in Mexico. However, based on all the consulted sources, several viral diseases associated with nine grapevine viruses have been reported in Mexico. These species have been grouped into seven genera and six families. The reports come from Aguascalientes (56%) and Baja California (44%). Three registered viral species are associated with the leafroll complex, three with rugose wood, one with fleck, one with infectious degeneration, and one with red blotch disease.

Findings/conclusions: Several grapevine viruses associated with major diseases have been reported in Mexico. Unfortunately, most of the reports lack detail and follow-up, and they are not readily available for international researchers; therefore, the lack of knowledge about this subject in Mexico is significant. Monitoring the epidemiology of viral diseases in the grapevine —a national and international relevant crop— is necessary.

Keywords: grapevine, viral diseases knowledge, agriculture.

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INTRODUCTION

The grapevine is a plant that belongs to the *Vitis* genera (Family: Vitaceae), which includes sixty native species from temperate regions in the northern hemisphere and some tropical regions. Twenty-five species can be found in North America alone. The most economically important species is *V. vinifera*, because its fruits are used to produce wine (about 68% of the production) and juices, as well as table grapes and raisins (Vaughan *et al.*, 2009). Grapevines are grown on more than 7,450,000 hectares around the world, which makes them the most economically important fruit-crop

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worldwide (OIV, 2016). There are about 10,000 cultivars of *V. vinifera*, and they can be classified according to color (red and white) or final use (table, wine, or raisins) (Vaughan *et al.*, 2009). In Mexico, in the 2018 agricultural cycle, 36,654.76 ha were sown with grapevines, obtaining a production of 444,446.87 tons, which amounts to over 9 million Mexican pesos (SIAP, 2020). The states with higher grape production percentages in Mexico are: Sonora (80.38%), Zacatecas (7.98%), Baja California (5.81%), Aguascalientes (3.48%), and Coahuila (1.09%). From the national production, 11% is used for raisins, 23% is used for industrial production, and 66% is used as table grapes (SAGARPA, 2018). Mexico produces 404,000 wine hectoliters, 12,000 of which are exported (OIV, 2016). Unfortunately, grapevine —just as other crops— can be impacted by different pests and pathogens. The problems that can have a high economic impact include viruses, some of which are highly pathogenic.

Nearly 80 virus species that can infect the *Vitis* genera have been identified, and around 25 grapevine viral diseases (Armijo *et al.*, 2016; Yepes *et al.*, 2018). About half of these viruses are associated with the four main complexes of the known grapevine diseases: infectious degeneration and decline, grapevine leafroll disease (GLD), rugose wood, and fleck disease. Additionally, there are recently discovered high-impact diseases such as the red blotch disease (Al Rwahnih *et al.*, 2013; Martelli, 2017). From the economic point of view, the most important grapevine viruses belong to the GLRaV complex (GLD-associated viruses), which are named in series (GLRaV-1 to -13) and can produce GLD in individual or mixed infections (Martelli *et al.*, 2012; Ito and Nakaune, 2016). Other important grapevine viruses are the grapevine virus A (GVA) and grapevine virus B (GVB), which belong to the *Vitivirus* genera. GVA causes Kober stem grooving, while GVB is associated with the corky bark symptom (Armijo *et al.*, 2016). Given that viruses cause significant economic diseases and that grapevine production is an important activity in Mexico, increasing the knowledge about grapevine virosis is necessary. Therefore, the objective of this study was to understand the situation of the grapevine viruses reported in Mexico. A literature review was carried out to expand the understanding about these viruses and the main impacted regions, as well as to share this information with the producers, in order to develop comprehensive control strategies.

MATERIALS AND METHODS

The information about the viruses reported in Mexico was obtained through a literature review, based on the data gathered from Scopus, Google Scholar, and EBSCO host, using the following words or keyword combinations: “virus”, “plant”, “grapevine”, and “Mexico”. Based on the lack of academic data resulting from the search using the abovementioned keywords, the databases of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) were also used, along with data gathered from undergraduate and postgraduate theses (Repositorio Institucional de CONACYT) and quotes from the consulted publications. The data was systematized by the taxonomy of the virus, the region where the virus was reported, and the disease complex to which the virus belongs.

RESULTS AND DISCUSSION

Information about grapevine viruses in Mexico

The results obtained from the search engines are reduced to just one file about a virus report in Mexico and a review. The first item is the initial report of the red blotch disease in Mexico; this disease is caused by the grapevine red blotch virus (GRBV) (Gasperin-Bulbarela *et al.*, 2019). The second item is a review of the said virus and its potential to spread the grapevine red blotch disease in Mexican vineyards (Beltrán-Beache *et al.*, 2021). The literature reviews also included international publications of interest about the subject that the search engines do not cover. These articles tackle the detection of corky bark and stem pitting symptoms, from which the term "madera rugosa" (the Spanish term for "rugose wood") was coined to describe plants that show these symptoms (Téliz *et al.*, 1980a; Téliz *et al.*, 1980b). The rest of the data obtained from the databases shows the recording of grapevine viral diseases associated with nine viral species in Mexico (Table 1).

Based on the limited data accessibility, five reports (56%) from Aguascalientes and four (44%) from Baja California were found; 50% of the viral species were reported in Aguascalientes, and 50% in Baja California (Figure 1A). These viruses are associated with different pathologies (Figure 1B). Additionally, in 2009 a technical brochure was published,

Table 1. Species of grapevine viruses documented in Mexico*.

Species	Family	Genus	Genome	Disease	Transmission
Grapevine fanleaf virus (GFLV)	*Secoviridae*	*Nepovirus*	ssRNA(+)	Fanleaf degeneration of grape	Nematode *Xiphinema index*, vegetative propagation, and grafting (Krebelj *et al.*, 2015).
Grapevine leafroll-associated virus 1 (GLRaV-1)	*Closterioviridae*	*Ampelovirus*	ssRNA(+)	Grapevine leafroll disease, (GLD)	Vegetative propagation (Rayapati *et al.*, 2014), mealybugs (Hemiptera: Pseudococcidae) and scale insects (Hemiptera: Coccidae) (Le Maguet *et al.*, 2012; Tsai *et al.*, 2010).
Grapevine leafroll-associated virus 3 (GLRaV-3)	*Closteroviridae*	*Ampelovirus*	ssRNA(+)		
Grapevine leafroll-associated virus 2 (GLRaV-2)	*Closteroviridae*	*Closterovirus*	ssRNA(+)		
Grapevine virus A (GVA)	*Betaflexiviridae*	*Vitivirus*	ssRNA(+)	Kober stem grooving	Grafting and by infected material (Yoshikawa, 2008), mealybugs and scale insects (Martelli, 2017).
Grapevine virus B (GVB)	*Betaflexiviridae*	*Vitivirus*	ssRNA(+)	Corky bark	Vegetative propagation and grafting (Maliogka *et al.*, 2015), mealybugs and scale insects (Golino *et al.*, 2002; Le Maguet *et al.*, 2012; Nakaune *et al.*, 2008).
Grapevine rupestris stem pitting-associated virus (GRSPaV)	*Betaflexiviridae*	*Foveavirus*	ssRNA(+)	Rupestris stem pitting	Vegetative propagation, grafting, and possibly via seeds (Gambino *et al.*, 2012).
Grapevine fleck virus (GFkV)	*Tymoviridae*	*Maculavirus*	ssRNA(+)	Fleck of grapevine	Infected propagative material (Martelli & Boudon-Padieu, 2006), no known vectors (Martelli, 2018).
Grapevine red blotch virus (GRBV)	*Geminiviridae*	*Grablovirus*	ssDNA	Grapevine red blotch disease	Grafting and propagative material (Cieniewicz *et al.*, 2018), *Erythroneura ziczac* Walsh y *Spisistilus festinus* (under experimental conditions) (Cieniewicz *et al.*, 2018; Poojari *et al.*, 2013).

* Not all reports specify the species; viruses associated with reported diseases are listed where appropriate. Taxonomy according to ICTV (2020).

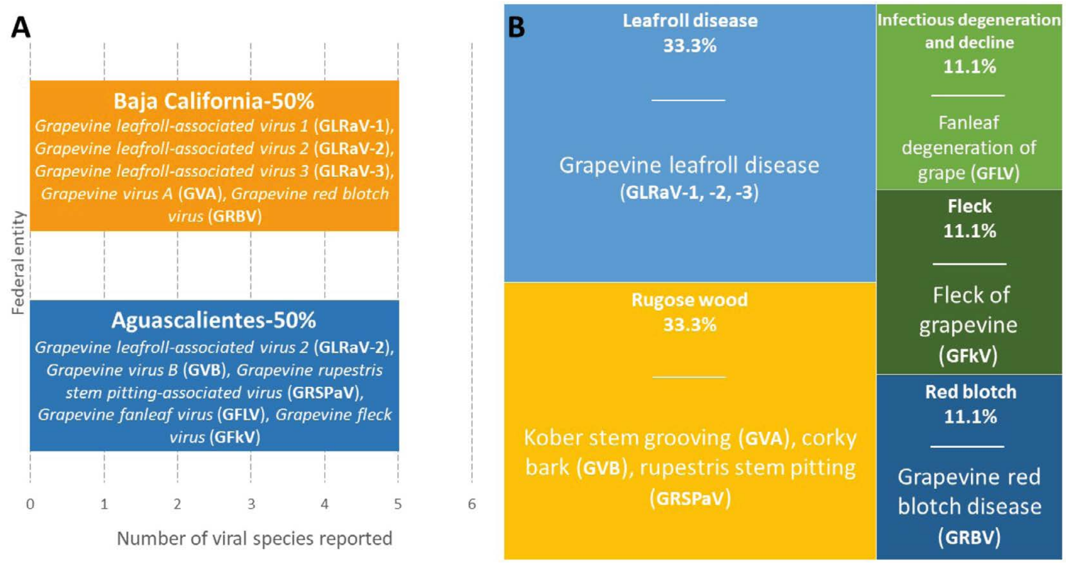

Figure 1. Reports of grapevine viruses in Mexico: A) Viral species by state (not all reports specify the species; viruses associated with reported diseases are listed where appropriate), B) Diseases and viral complexes.

including recommendations about the handling of crown gall and viral grapevine diseases in Zacatecas. Emphasis is placed on the viruses of the grapevine leafroll virus complex (GLRaV) and the grapevine fanleaf viruses (GFLV) (Velásquez-Valle *et al.*, 2009), probably because those viruses were already present in that region. There were also undergraduate virosis theses (Monroy-Corral, 2019; Palacios-Gutiérrez, 2019). However, none of the search engines used for the research included them among the results. Therefore, other similar works might not have been included in the search results, because they are not readily available.

Chronology of grapevine viruses reports in Mexico

Téliz *et al.* (1980b) reported that, in 1968, grapevines with disease-free stocks were sown in the Estación Experimental Agrícola CIANOC-Pabellón of Aguascalientes. In 1970, some of these plants started to show a curly and reddish or yellowish foliage. The disease continued to spread until 1978, and almost all the plants had stem pitting symptoms. Therefore, the authors sought to determine the relationship between the symptoms of the already known grapevine diseases, their distribution, their effects on commercial vineyards, and the development of those symptoms during the establishment of the vineyard. Corky bark and stem pitting symptoms were detected in 103 out of the 311 vineyards inspected —in 75% of the said vineyards, 4,593 plants showed stem pitting symptoms, and 3,728 showed corky bark symptoms— and in 21 out of 32 grapevine cultivars. Cardinal, Malaga Champagne, Exotic, Tokay, San Emilion, Chardonnay, and LN-33 indicator plants were the most impacted cultivars. Corky bark and stem pitting symptoms were detected in five cultivars, fluctuating from 53 to 64% and 68 to 84%, respectively. However, they were not associated with any specific virus. Additionally, the term "madera rugosa" was proposed for a set of symptoms, which included pitting and longitudinal grooves in the stem and the basal internodes of the sprouts (Téliz *et al.*, 1980a). The data was not conclusive regarding the disease found: the plants showed both corky bark and stem pitting symptoms, which led the researchers to conclude that both symptoms were related to the same disease. In the 1970s, corky bark and rugose wood symptoms were detected in Salvador, Carignan, Superior, and Red Glove cultivars in two vineyards of Aguascalientes (Téliz *et al.*, 1980b). Valle and Téliz (1983) reported that the incidence of the corky bark varied from 64 to 84% in cultivars such as Exotic, Tokay, and Red Malaga.

The GVB, grapevine rupestris stem pitting-associated virus (GRSPaV), GVA, and grapevine virus D (GVD) belong to the rugose wood diseases complex. These viruses can cause four distinct disorders: rupestris stem pitting, corky bark, Kober stem grooving, and LN33 stem grooving (Moradi *et al.*, 2018). The corky bark damage is attributed to the infection by GVB, a virus of the *Vitivirus* genera (Boscia *et al.*, 1993; ICTV, 2020). For its part, GRSPaV has been associated with rupestris stem pitting (Martelli, 1993). Therefore, the two species were likely present since the vineyards were established, because at least GVB was later identified in the region. From 2007 to 2009, research work was carried out in two commercial vineyards from Aguascalientes, in order to quantify the incidence and severity of corky bark, with various cultivar and rootstock combinations, including Red Globe/Franco, Superior/Franco, Red Globe/Salvador, and Salvador/1103-P. The researchers determined a 27.5-66.1% incidence of plants with corky bark-rugose wood symptoms in one vineyard and up to an 81.6% incidence in the other. GVB and the grapevine leafroll-associated virus 2 (GLRaV-2), one of the causative agents of GLD, were identified using an RT-PCR protocol (Velásquez-Valle *et al.*, 2010). Most of the varieties and rootstocks of the infected plants came from Ojocaliente, Zacatecas, although not necessarily from the same vineyard; they had originally been obtained from the USA, as "virus-free" material. Additionally, as a result of monitoring carried out in 2006, symptomatic plants were detected, and GVB was identified in SH-3 and Dog Ridge rootstocks, along with grapevine fleck virus (GFkV) in SH-3 rootstocks (Velásquez-Valle *et al.*, 2010).

In 2013, random samples were collected from 12 out of the 22 commercial vineyards in Aguascalientes to determine the incidence of viral diseases. The DAS-ELISA analysis of the samples from the plants collected —which included the Chenin Blanc, Chenin Blanc/Dog Ridge, Salvador Franca, Red Globe Franca, Red Globe/Rupestris, and Salvador varieties— revealed the presence of the grapevine fanleaf virus (GFLV), with a 6.7-37.5% incidence in the Chenin Blanc, Red Globe/Rupestris, and Salvador varieties. Samples from 5 out of the 12 vineyards tested positive for the virus (Velásquez-Valle *et al.*, 2013). GFLV is the causative agent of the infectious degeneration of the grapevine leaf that has spread throughout the world (Andret-Link *et al.*, 2004). The DAS-ELISA serological analysis carried out by Velásquez-Valle *et al.* (2013) in Aguascalientes also recorded the presence of a non-specified GLRaV in the Chenin Blanc, Chenin Blanc/Dog Ridge, Salvador Franca, Red Globe Franca, and Salvador varieties, with a 3.8-80% incidence. The authors suggest that the original infected plant material is the primary contamination source.

Meanwhile, evaluating the potential regional vectors of viral diseases is highly important. The main vectors for GLRaVs are mealybugs (Hemiptera: Pseudococcidae) and scales (Hemiptera: Coccidae). Vine mealybugs (*Planococcus ficus*) were first detected in

Baja California in 2014 (CESVBC, 2018); subsequently, samples from plants with GLD symptoms were collected in 2018. Following RT-PCR protocols, the GLRaV-1, GLRaV-2, and GLRaV-3 viruses were first detected in Baja California, along with GVA —another virus transmitted by vine mealybugs. GLRaV-1 was the dominant GLRaV virus (26%), followed by GLRaV-3 (24%) (Monroy-Corral, 2019). At the same time, massive sequencing techniques (RNA-seq) and bioinformatics analysis were used to identify and search for viruses with RNA or DNA genomes. The aim was to know the viruses found in *P. ficus*, in order to propose alternatives to control the insect populations. According to the results, the presence of one or more putative RNA viruses which could be specifically associated with *P. ficus* has been detected, as well as another virus potentially associated with the plant; however, their characterization is an ongoing effort (Duarte-De Jesús, 2020; Martinez-Mercado *et al.* 2022).

The grapevine plant is frequently co-infected by one or more GLRaV species, in addition to GVA and GVB (Le Maguet *et al.*, 2012); therefore, when plants show inconclusive symptomatology, various viruses should be subject to a joint monitoring. A clear example of this problem was the discovery in 2008 of the red blotch disease in plants showing GLD-like symptoms, in a Cabernet Sauvignon vineyard at the Oakville Station of the University of California (Calvi, 2011). GRBV is now known to be the cause of this disease (Sudarshana *et al.*, 2015; Cieniewicz *et al.*, 2017). The first report about the presence of GRBV in Mexico was made in 2019 (Gasperin-Bulbarela *et al.*, 2019). Throughout 2016 and 2017, samples with amplification of the expected products were found by PCR-based diagnosis of *Vitis vinifera* Pinot noir, Merlot, and Nebbiolo plants which showed red blotch symptoms at Ensenada, Baja California. A representative amplicon sequence had a high identity (98%) with Canadian GRBV isolates. Likewise, Gasperin-Bulbarela *et al.* (2019) reported the full sequence of two new Mexican GRBV isolates. In a follow-up work, in 2018 and 2019, they carried out a sample focused on plants with reddening symptoms in Baja California and found positive samples in red and white varieties, using PCR. The main symptoms in plants from positive red varieties were red blotches in the leaf blade and the secondary and tertiary red veins, while foliar chlorosis was recorded in white varieties. Additionally, an analysis of the viral DNA restriction patterns of 21 positive samples enabled the identification of isolates from Washington, New York, California, and Canada; this suggests several points of origin or entry paths for GRBV (Palacios-Gutiérrez, 2019). Beltrán-Beache *et al.* (2021) reviewed the current global situation of the red blotch virus and the risk it entails for Mexico, and they emphasized the lack of knowledge about the incidence and geographical distribution of the virus in the country. The only official report about the situation was developed by Gasperín-Bulbarela *et al.* (2019). However, those authors do not discard the presence of the virus in other areas; therefore, detecting the disease is fundamental to avoiding its transmission.

CONCLUSIONS

According to our review, the following viruses have been reported in Mexico: GFLV, GLRaVs, GVA, GVB, GRBV, GFkV, and GRSPaV. Most of those reports lack detail and follow-up. Although grapevine is a highly important crop for the country, there is scant information about viral diseases. Additionally, none of the reports we reviewed evaluated the economic impact of the diseases; such data should be transferred to the producers and technicians who manage the vineyards. In general terms, this information is not readily available, which hinders the appreciation and knowledge of the contributions made by students and others parties interested in the area. Therefore, we strongly suggest publishing information in formal media (particularly international media), in order to provide easy access for members of the academia and the industry. The management and characterization of grapevine viral diseases are a highly complex process, due to their chronic nature and their symptomatology, which may change from one grapevine variety to another. Therefore, accessible, quick, and sensitive new diagnosis techniques are required. Likewise, integrated disease management would benefit from establishing interactions between members of the academia and producers, facilitating management decision-making.

Mexico lacks significant knowledge about viral infections of grapevine; therefore, much work is to be done. Some potential initial steps could include: studying viral epidemiology in grapevines; carrying out accurate diagnoses in places where suspicious (symptomatic) plants have been detected; monitoring areas where viruses have been detected in the past; identifying and follow-up current vectors; and studying the effects of viral infection in plants and the economic impact on crops. Additionally, the potential co-infection of plants, the impact of mixed infections in crops, and the agents that take part in transmission could be addressed. Both the collection of field data and the application of molecular biology tools help to understand the etiology of viral diseases, as well as the host-pathogen-vector interaction.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Corn (*Zea mays* L.) green matter production in different sowing densities

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ABSTRACT

Objective: To evaluate the corn green matter production at different sowing densities, in Valle del Mezquital, Hidalgo, Mexico.

Design/Methodology/Approach: The DK-4018 hybrid corn was sown in the spring-summer 2021 cycle. The treatments (T) were different sowing densities: (T1) 112,500, (T2) 120,000, and (T3) 136,000 plants per hectare. The experimental design was completely randomized. The evaluated variables were: plant height at 30, 60, and 76 days after sowing (cm); final stem diameter at ground level (cm); fresh weight (g); and green matter yield (ton ha^{-1}). The results were analyzed with the SAS program (2001) and the means were compared with the Tukey test ($p \leq 0.05$).

Results: The highest green matter production of the DK-4018 hybrid corn was obtained with a sowing density of 136,000 plants per hectare, with an estimated yield of 114.6 ton.

Study limitations/implications: No scientific publications about the DK-4018 hybrid corn green matter production were found.

Findings/Conclusions: With a population density of 136,000 corn plants per hectare, 114.6 ton of DK-4018 corn green matter yield is estimated.

Keywords: production, fresh weight, plant height, Valle del Mezquital.

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INTRODUCTION

Corn (*Zea mays* L.) a plant native to Mexico and Central America— is, after wheat and rice, the most important crop in various parts of the world and has adapted to different climatic and soil conditions. It is mainly used for human consumption and as animal feed (grain or fresh forage), as a result of its high aerial biomass content (35 to

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95 tons per hectare) (Sánchez *et al*., 2013). In the dairy basins of Mexico, corn forage is mainly used to feed dairy cattle (Zaragoza *et al*., 2019). It is one of the major crops in the world in terms of sown area each year: 8.5 million hectares for grain on average, 25% of them sown with improved seed, and 75% with native seeds (Tadeo *et al*., 2016). In industrialized countries, it is mainly used to produce processed foods and, recently, ethanol. Its yield and quality depend on soil fertility, crop management, and its genetics. It is considered an excellent ruminant feed, due to its high energy and protein content (Santiago *et al*., 2018). Currently this grass is the crop with the greatest diversity of uses, applications, forms, and production conditions. Producers consider the corn plant to be excellent fresh or dry forage. A higher crop density is needed for forage production than for grain production. Consequently, 30,000-90,000 plants ha^{-1} sowing densities have been recommended, depending on the region, irrigation, genotype, and fertilization level, although a greater amount of biomass has been obtained in other trials, using 73,000 to 80,000 plants ha^{-1} (Vázquez *et al*., 2013; Sánchez *et al*., 2019). The objective of this research was to evaluate the corn green matter production at different sowing densities, in Valle del Mezquital, Hidalgo, Mexico.

MATERIALS AND METHODS

This research was established in the spring-summer 2021 cycle at the experimental platform of the Universidad Politécnica Francisco I. Madero, located in the Francisco I. Madero municipality, in the state of Hidalgo, Mexico. The town is located at 1,995 masl, 20° 15’ 20” N and 99° 00’ 10” W. It has a cold temperate climate, a 17 °C average annual temperature, and a 540 mL annual rainfall (Rodríguez-Ortega *et al*., 2013).

The DK-4018 (Dekalb Genetics Corporation) hybrid corn was sowed in a plot that had been previously prepared using traditional cultural practices. The treatments were different sowing densities (DDS): T1=112,500, T2=120,000, and T3=136,000 plants per hectare, on a surface with homogeneous climatic and edaphic conditions. The experimental design was completely randomized with five repetitions of 4 m^2 per experimental unit, using a five diagonal point sampling method. Several agronomic activities —including fallowing, soaking, and harrowing— were carried out before sowing to obtain optimal crop development. The corn seed was placed in the hoppers of the following planters: a 1035 John Deere model for T1 and a Magnus Dobladense model for T2 and T3. A granulated fertilizer mixture with a 22N-08P-12K formula was added and micronutrients were applied 32 days after sowing. Two gravity irrigations were carried out at 30 and 50 days, until the first rainfalls took place. To avoid weed competition, a selective post-emergence herbicide was applied. The variables measured in the development of the crop were plant height at 30, 60, and 76 days after sowing; final stem diameter at ground level (cm), fresh weight (g), and estimated yield of green matter (ton ha^{-1}) were also measured at 76 days. The results were analyzed with the SAS program (2001) and the means were compared with the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Sanchez *et al.* (2013) mention that corn sowing densities vary according to the production objective (grain, forage, or both); they recommend an optimum population density of 39,520 to 98,800 plants per hectare for forage corn, because in theory the total forage biomass increases proportionally to plant density. It is also known that the optimal density of corn plants for grain and forage yield depends on the type of hybrid, soil fertility, and agronomic management of the crop. Therefore, when forage corn was evaluated at a density of 104,000 plants per hectare under irrigation conditions, forage yields between 27.8 and 70.2 t ha^{-1} were obtained. Hybrids such as H-376 —which are used to produce grain and forage— should be sown under irrigation, with an 80,000 plants ha^{-1} density, in order to obtain an estimated green matter yields of 78.1 to 90.8 t ha^{-1}. Forage corn yields of 52.5 to 85.6 t ha^{-1} were obtained with Aspros-721, H-31, VS-2000, and cacahuacintle at 85,000 plants ha^{-1}.

Plant height. Table 1 shows the average plant height results at 30, 60, and 76 days after sowing. There is no statistical difference ($p>0.05$) at 30 days between the three treatments (Figure 1). The value obtained 60 days after sowing did show significance; the best averages were obtained with T2 and T3 (169.9 cm). At 76 days after sowing, plant height was also significant ($p\leq0.05$); T2 produced the tallest plants (277.5 cm).

Table 1. Plant height at 30, 60, and 76 days after sowing, stem diameter, fresh weight and green matter yield of DK-4018 sown at different sowing densities, in the cycle spring-summer in Tepatepec, Francisco I. Madero, Hidalgo, Mexico.

Variables	Tratamientos (DDS=plantas ha^{-1})		
	T1: 112,500	T2: 120,000	T3: 136,000
Altura a los 30 días (cm)	25.300 a	26.300 a	28.200 a
Altura a los 60 días (cm)	144.900 b	169.900 a	169.900 a
Altura a los 76 días (cm)	247.700 b	277.500 a	271.200 a
Diámetro de tallo (cm)	2.500 b	2.600 a	2.600 a
Materia verde (g $planta^{-1}$)	689.320 b	880.200 a	842.800 a

Values with different letters in each row are statistically different (Tukey, $p\leq0.05$). DDS=sowing density.

Figure 1. Corn crop evaluated at different sowing densities. T1=112,500, T2=120,000, and T3=136,000 plants per hectare.

Marcos *et al.* (2016) report similar stem heights in native corn (average value: 270 cm).

Plant height recorded 30 days after sowing was not significant ($p>0.05$) between treatments (Table 1). These results are like those obtained by Quispe *et al.* (2011), who recorded average 28.19 cm heights in Testigo Joya and Testigo Canta corn. In this study, the results showed that, 30 days after sowing, the corn plants did not compete for space, water, light, and nutrients in the three densities of the experiment, because the preexistent soil conditions cover those needs. At 60 days after sowing, a statistical difference was found in treatments T2 and T3, with higher values (Table 1).

Significant differences in corn plant height were observed ($p\leq 0.05$) at 76 days after sowing (Table 1). Rodríguez and Rabery (2003) evaluated the distance between furrows in two sowing times to obtain higher grain yield and reported similar results, concluding that corn plant height is determined by both genes and environmental conditions. Cervantes *et al.* (2014) analyzed the effect of population density and corn genotypes, observing that the increase in population density generally results in larger plants, which is consistent with the results of this research. Other studies about the same subject also evaluated the effect of three sowing densities in four purple corn genotypes yields and concluded that they did not affect plant size. Meanwhile, Antuna *et al.* (2003) evaluated the agronomic behavior of six corn lines and their hybrid combinations and highly recommend using short-statured materials that tolerate high densities and have resistance to stalk lodging, without neglecting the positive relationship between plant height and grain yield potential.

Stem diameter. Stem diameter at 76 days after sowing was different between sowing densities ($p\leq 0.05$): the lowest sowing density (T1=112,500 plants per hectare) resulted in smaller diameter stems. This probably helps to avoid stalk lodging problems (Table 1). These results are similar to the findings of Sánchez *et al.* (2013), who obtained an average stem diameter of 2.33 cm when evaluating 24 commercial hybrid corns, under adequate soil, temperature, and precipitation conditions.

Intriago and Torres (2018) analyzed the effect of sowing density and crop arrangement on the growth and development of the HAZ1 corn hybrid; they report that there were no significant differences, neither as a result of the effects of the sowing arrangement and population density, nor of the interaction of the arrangement and density regarding plant height, stem diameter, and root weight. They also mention that an increase in population density significantly affects plant height, while stem diameter decreases due to competition for light. Additionally, Cruz (2017) evaluated the effect of four sowing densities and four fertilizer doses in the development and yield of corn and determined that the diameter and height of the plant have significant differences in the density factor. Likewise, she reports that, as the density increases from 50,000 to 126,000 plants per hectare, the corn plant stem diameter decreases, and that stem diameter can be affected by high sowing densities and competition for light, causing stalk elongation and consequently diameter reduction. She also reports that plants at low densities per hectare (50,000 plants ha^{-1}) reached the same height as plants at medium densities (76,000 plants ha^{-1}); on the contrary, high densities (101,000 plants ha^{-1}) presented greater height than plants at low densities. Therefore, she concluded that at high densities the diameter is reduced, and the plants elongate.

Green matter weight. There were statistical differences in the production of green matter per plant (Tukey, $p \leq 0.05$; Table 1). T2 (120,000 plants ha^{-1}) has the greatest weight with an average of 880.20 grams $plant^{-1}$, exceeding the green matter recorded in T1 (112,500 plants ha^{-1}) by 22.3%. Similarly, Vásquez (2019) reported that sowing density determines the differences in fresh weight of corn plants. Cusicanqui and Lauer (1999) determined that the dry matter yield increased at a density of 97,300 and 102,200 plants per hectare. In contrast, Santiago *et al.* (2018) report low yields of green matter per hectare of corn and point out that soil fertility, crop management, and genetics are important factors to obtain good yields. For their part, Wiersma *et al.* (1993) and Guyader *et al.* (2018) report that climatic conditions (*e.g.*, precipitation and drought) are major factors that determine the good development and yield of the crop.

Yield. The green matter yield per hectare of DK-4018 corn was statistically different between sowing densities ($p \leq 0.05$) at 76 days after sowing. The best results were obtained with the two highest densities (Figure 2); under the experimental conditions of this study, the sowing density and fresh weight yield per treatment have a directly proportional relationship.

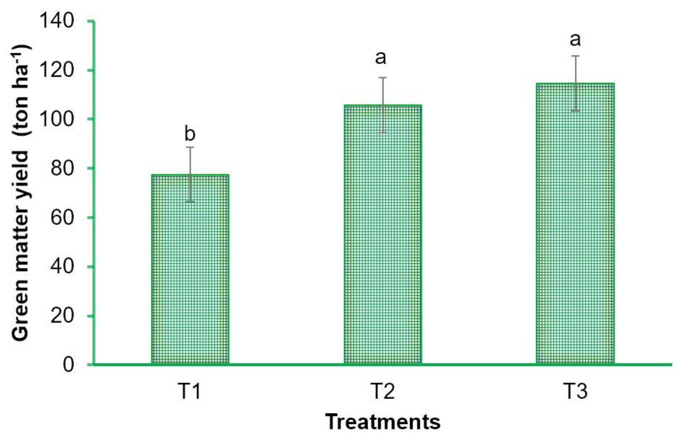

Figure 2. Green matter yield of DK-4018 corn with three sowing densities. Bars ± DE with different letters are statistically different (Tukey, $p \leq 0.05$). DDS=sowing density. T1=112,500, T2=120,000, and T3=136,000 plants per hectare.

CONCLUSION

For green matter production, the best sowing density of the DK-4018 corn hybrid is 136,000 plants per hectare, with an estimated fresh weight yield of 114.6 tons, under the edaphic and climatic conditions of Valle del Mezquital, Hidalgo, Mexico.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Long term orientation: A comparative study amongst engineer and tourism students

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ABSTRACT
Objective: To investigate differences amongst the LTO (Long Term Orientation) profile of graduate students of two academic programs: tourism and engineer.
Design/methodology/approach: A total of 66 students participated in the study. The items of each construct corresponded to the two dimensions established by the original LTO scale. The validity test for the measurement scale was based first on exploratory and then on confirmatory factor analysis. The internal reliability consistency of the multi-item scales was assessed with Cronbach's alpha. Independent sample t-tests were applied to verify the hypothesis.
Results: The eight-item LTO scale performed reasonably well, lending support for its internal validity for the sample. The engineering students (6.16 ± 0.65) had higher levels for the planning dimension compared with the tourism students (6.10 ± 0.56), still there were no significant differences in the estimates ($t=-0.391$, $p=0.697$), and students of the tourism program rated significantly higher ($t=3.557$, $p=0.001$) for the tradition dimension (6.12 ± 0.59) compared to the engineering students (5.42 ± 0.90).
Limitations/implications: The study focus only in one personality trait. Education providers can draw upon these findings a better understanding of their students, becoming relevant for the curriculum.
Findings/Conclusions: Students of the tourism academic program score higher in the tradition dimension of the LTO profile. On the contrary, there was no difference regarding the planning factor of the LTO profile. Therefore, LTO scale might be useful for understanding students' decisions and personal orientations, allowing for academic programs to better focus their curriculum.

Keywords: Tourism education, engineer students, student profile, construct validity, planning, tradition.

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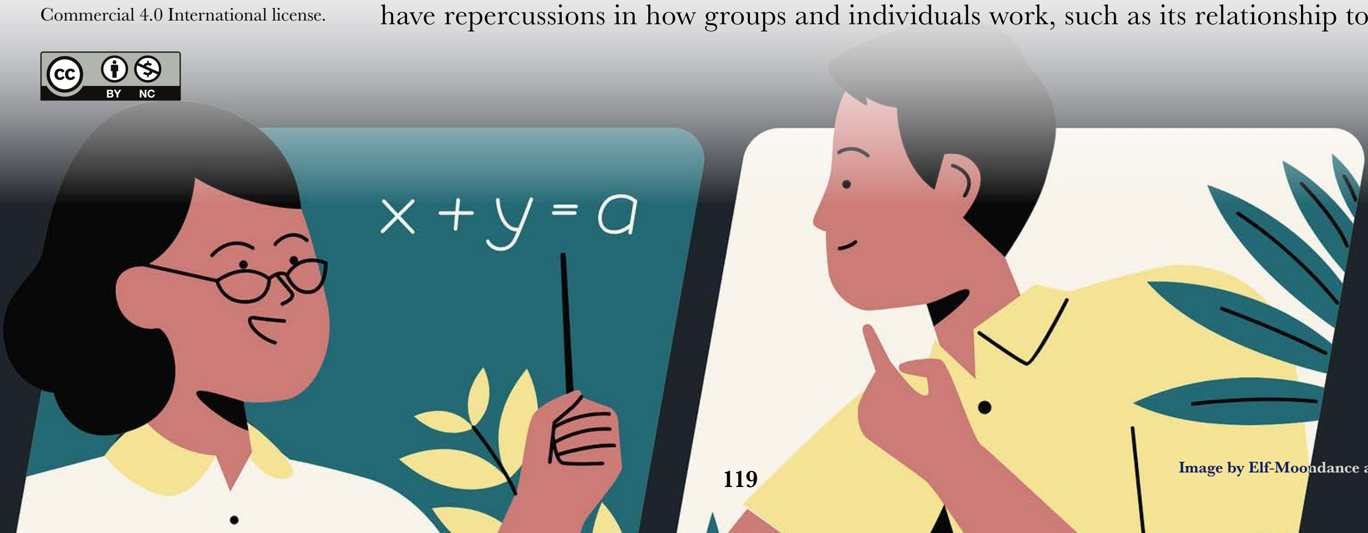
INTRODUCTION

Many studies have shown the relevance of long term orientation (LTO) amongst people as a cultural trait (de Mooij and Hofstede, 2010). National cultural models are patters that have repercussions in how groups and individuals work, such as its relationship towards



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authority, self-conception and conflict dilemmas and how to solve them (Kluckhohn and Strodtbeck, 1961). One of the cultural dimensions most studied has been the long and short them orientation, which is the measure by which a society exhibits a pragmatic perspective towards the future instead of an historical view or a conventional short term one. The values considered in the long term orientation (LTO) are: perseverance, status, saving and having a sense of shame. The contrary is the short term orientation that includes: firmness and personal stability, as well as respect for tradition. The attention is placed on the search of happiness more than mental peace. The long term orientation implies investing on the future (de Mooij and Hofstede, 2010). It can be said that the conceptualization of the long term orientation is a prospective vision, meaning by that a long term or future one; still, it also considers a past vision, that is to say a short term, with two sub-dimensions: tradition and planning (Arli and Tjiptono, 2014). Long or short term orientation has implications in the individual's actions, as those that present a long term orientation will seek productivity whist those with a short term orientation will focus on tradition.

One of the main critics to the national culture models is that they are based on a study carried out as an aggregated level and not an individual level, therefore, values could be attributed to individuals or groups and not of a national culture or subculture. Due to this criticism, Bearden *et al.* (2006), developed and validated a measure to evaluate short and long term orientation regarding time in a scale known as long-term-orientation (LTO), which has two dimensions: planning and tradition.

The effect of the individuals LTO has been previously analyzed in different studies, such as: entrepreneurial behavior of family businesses (Lumpkin *et al.*, 2010); customer relationships management (Olavarria-Jaraba *et al.*, 2018); and, ethical values (Nevins *et al.*, 2007). Some studies followed a gender approach (Sreen *et al.*, 2018), others focused on the national level (Fang *et al.*, 2013; Lortie *et al.*, 2019). Many studies are from the marketing area (Abubakar and Mokhtar, 2015; Olavarria-Jaraba *et al.*, 2018), whilst others are more interested in understanding its effects in business management (Ryu *et al.*, 2007; He and Sun, 2020).

LTO and education, has also been studied previously. For example, Fang *et al.* (2013) found that national culture played a role in the success or lack of it of young learners. Rodríguez-Gázquez *et al.* (2021), compared national values amongst nursing students of Spain and Colombia, noticing that there were significant differences due to country culture. Whilst Cidral *et al.* (2020) found that students' long-term orientation positively influences the use of e-learning systems' and its net-benefit perception. Therefore, LTO seems to be relevant for understanding students' decisions and performance (Kvan and Jia, 2005).

To the best of our knowledge no literature in the Mexican context has studied LTO amongst graduate students of different orientation programs. This research intends to cover this gap by applying LTO scale to compare students belonging to two different graduate programs: tourism and engineering. Our aim in this research is to investigate the differences amongst the LTO profile of graduate students in two academic programs. Our findings will benefit academic programs to better focus their curriculum.

Conceptual background and hypothesis development

To assess time orientation, Bearden *et al.* (2006) developed and validated LTO, a scale that assess differences in long and short term orientations regarding time, a main difference with other scales is that this has a holistic view of the future and the past and no merely focuses on "here and now". Therefore, viewing time holistically can be considered as a cultural value. The scale is constructed as a two-factor, where tradition and learning from the past is considered as the short-term value and persistence is the long term value.

Academic orientations and LTO

Students on the engineer area are formed considering their abilities to generate research as well as being able to develop innovations in their area, following a science-oriented program (Valdés-Cuervo *et al.*, 2013). This discipline learning process is focused on logical and analytical thinking strategies and reproduction directed learning whilst social sciences, where tourism students belong, learning process focus on internal motivation, critical and holistic thinking strategies as meaning-directed learning (Vettori *et al.*, 2020). As the learning process as well as the academic paths are different for tourism and engineering graduate students, we state that their LTO profile should also be significantly different. As we used the Bearden *et al.* (2006) scale, which has two factors: tradition and planning, therefore we hypothesize that:

H1. Students of the engineering academic program might score higher in the planning factor.

Tourism is one of the most important industries in the world (Lu and Adler, 2009), still, tourism education suffers from poorer academic intakes often combined with low levels of aspiration and performance, which leads to low long-term engagement with careers in the industry. Tourism studies normally have lower entry levels than other subjects. And the choice to study tourism, is related to a personal interest in travel and personal circumstances and convenience, with little attention to subject relevance (Ramakrishnan and Macaveiu, 2019). Thus, we hypothesize the following:

H2. Students of the tourism academic program might score higher in the tradition factor.

MATERIALS AND METHODS

Sample and data collection

Data derived from a convenience sampling at one public graduate school campus in Veracruz, Mexico. Students taking a business course first responded the questionnaire, then they were asked to help in applying the questionnaire to their peers on campus in classrooms and public spaces. The students belonged to two programs offered at the same campus, the tourism program has a more practical application whilst the engineering program is research oriented. The first program has 13 generations of graduate students and the other one 18 generations, each generation has between 3 and 10 students, being the tourism program the less populated, students belonging to four different generations participated in the study. A total of 66 students, mostly females, participated in the study (Table 1). The gender distribution is related to the overall distribution on both programs, which are mainly female students. No student of the two programs refused to take part in the research.

Measurement scales

All the measures used in this study were drawn from existing literature and adapted to serve the purpose of this study. The items were based from Bearden *et al.* (2006), they were translated from English into Spanish and using the back translation method to ensure the reliability and concordance of the translation process. The questionnaire had only two parts, the first were some basic demographic information, the second was the LTO section. It was adjusted by discussing it with five experts in the business area, and a pilot was tested with five engineering students. The items of each construct corresponded to the two factors established by the original LTO scale (Table 2). The responses were sought on a 7-point Likert scale ranging from 1 (Strongly disagree) to 7 (Strongly agree).

Table 1. Demographic of the sample (%).

Demographic	Tourism (n=27)	Engineering (n=39)	Total (n=66)
Gender			
Male	11.11	35.90	25.76
Female	88.89	64.10	74.24
Age			
21-26 years	48.10	59.00	54.60
27-31 years	33.40	30.80	31.80
32-36 years	7.40	5.10	6.10
37-46 years	11.10	5.20	7.50

Table 2. Items of the LTO scale.

Item	Total (n=66)		Tourism (n=27)		Engineering (n=39)	
	Mean	SD	Mean	SD	Mean	SD
Respect for tradition is important to you	5.98	1.21	6.48	0.13	5.64	0.22
You plan for the long term	5.82	0.83	5.74	0.15	5.87	0.14
Family heritage is important to you	5.44	1.52	6.19	0.17	4.89	0.27
You value a strong link to your past	5.40	1.31	5.63	0.22	5.22	0.24
You work hard for success in the future	6.42	0.66	6.33	0.12	6.49	0.11
You don't mind giving up today's fun for success in the future	5.88	1.23	5.85	0.22	5.89	0.21
Traditional values are important to you	6.03	0.88	6.19	0.15	5.92	0.15
Persistence is important to you	6.45	0.71	6.48	0.14	6.44	0.11

Data analysis

The information gathered was analyzed with the SPSS version 20.00 program (SPSS Inc., Chicago, IL, USA). A conventional validity and reliability tests were conducted (Clark and Watson, 2019). The validity test for the measurement scale was based first on exploratory and then on confirmatory factor analysis (CFA). The internal reliability consistency of the multi-item scales was assessed with Cronbach's alpha (Bearden *et al.*, 2006), in some cases average variance extracted (AVE) (Fornell and Larcker, 1981), and composite reliability (CR) (Vinzi *et al.*, 2010) values, were calculated. Independent sample t-tests were applied to verify the hypothesis.

RESULTS AND DISCUSSION

LTO for all students

In order to check for cross-loadings and to replicate the analyses of Bearden *et al.* (2006) an exploratory principal-axis factor analysis with varimax rotation was undertaken on the data for both program samples. The Kaiser-Meyer-Olkin value was 0.652, therefore, the application of the factor analysis was sufficient for these data set. In Table 3, results for both programs are presented. The first factor extracted was planning.

In this initial study, dimensions seemed to be similarly as conceived by Bearden *et al.* (2006). The eight-item LTO scale performed reasonably well, lending support for its internal validity for the sample, including students of both programs.

LTO for each academic program

When an exploratory principal-axis factor analysis with varimax rotation was undertaken on the data for each program samples, data did not behaved as established by Bearden *et al.* (2006).

In the case of the students of the engineering program, three factors were found, the first one with the planning dimension, the second one had the tradition dimension, except

Table 3. Long-term orientation items with factor loadings and reliability values.

Item	Component 1	Component 2
Tradition		
Respect for tradition is important to you		0.658
Family heritage is important to you		0.631
You value a strong link to your past		0.782
Traditional values are important to you		0.636
Planning		
You plan for the long term	0.681	
You work hard for success in the future	0.845	
You don't mind giving up today's fun for success in the future	0.604	
Persistence is important to you	0.749	
Initial eigenvalues (29.92% variance explained)	2.394	
Extracted components (52.40% variance explained)	1.798	
Cronbach's alpha	0.624	0.626

for the item: "Family heritage is important to you"; that became another dimension (0.922) with "You value a strong link to your past" (0.676). The Kaiser-Meyer-Olkin value was 0.541, the initial eigenvalues (2.316) explained 28.94% variance, the extracted components (1.110) the 67.13%. However, the average variance extracted (AVE) value met or exceeded the recommended level of 0.5 for components two and three, and component one value was 0.44, still the composite reliability (CR) values for all constructs were all above 0.6. (Fornell and Larcker, 1981).

The tourism program sample had two components, the first one with six factors, and the second one was composed by the items: "Respect for tradition is important to you" (0.826) and "Traditional values are important to you" (0.787). The Kaiser-Meyer-Olkin value was 0.696, the initial eigenvalues (3.136) explained 39.19% variance, the extracted components (1.458) the 57.41%. Both AVE and CR values were adequate (Fornell and Larcker, 1981).

Differences in the number of dimensions can be attributed to the sample size (Kyriazos, 2018), but also, it can mean that the planning dimension was more solid contrary to the traditional one. This might be because both populations were students. The third component in the cultural dimension for the engineering sample can be attributed to the fact that the item "Family heritage is important to you" scored the lowest, distancing itself of the rest of the items in that dimension, therefore behaving as a different latent variable (Ziegler and Hagemann, 2015).

Academic orientations and LTO dimensions

Paired t-test were then computed to examine the difference between estimates of LTO dimensions: tradition and planning, and academic programs: tourism and engineering (Table 4). Though there was a small difference between both programs, with the engineering students with higher levels for the planning dimension, there were no significant differences in the estimates ($t=-0.391$, $p=0.697$). Therefore, results did not support H1. On the contrary, tests of H2 supported the predictions, as students of the tourism program rated higher for the tradition dimension compared to the engineering students ($t=3.557$, $p=0.001$).

Previous studies have shown that academic majors do influence students intentions because the knowledge it is provided to the students (Dao *et al.*, 2021), also, students of social sciences tend to have learning process focused on internal motivation more than being focused on local and analytical thinking as students of the technical sciences do (Vettori *et al.*, 2020). In this case, the students of the tourism program are oriented to understanding and preserving tradition, which is consistent with our findings.

Table 4. Dimensions of the LTO scale.

Dimension	Total (n=66)		Tourism (n=27)		Engineering (n=39)	
	Mean	SD	Mean	SD	Mean	SD
Tradition	5.70	0.86	6.12*	0.59	5.41*	0.90
Planning	6.13	0.61	6.10 ns	0.56	6.16 ns	0.65

*: T-test, $p<0.05$; ns: not significant.

Limitations and future research

One of the limitations of this studies is that if focus only in one personality trait: long term orientation; whereas other studies have showed that variables such as gender, learning approaches (Vettori *et al.*, 2020), ethics (Nevins *et al.*, 2007), could give a more holistic understanding of the students' decisions and performance. Therefore, a recommendation for further research would be to include other variables in the study.

Another limitation of this study was the sample size, which might influence the results, especially when each program was analyzed independently. This sample is justifiably as the general population of students in the program is small (between 4 and 10 per generation), therefore almost four generations participated in the study. Future studies with larger populations of students might produce more generalizable results. A study considering undergraduate students may yield interesting differences and similarities regarding long term orientation.

CONCLUSIONS

In this research, we have explored the differences amongst the long term orientation (LTO) profile of graduate students in two academic programs: tourism and engineering. Using empirical data from students of two graduate programs, we tested two hypotheses. Results support the proposed effects: Students of the tourism academic program score higher in the tradition factor of the long term orientation profile. On the contrary, there was no difference regarding the planning factor of the long term orientation profile. Therefore, LTO scale might be useful for understanding students' decisions and personal orientations, allowing for academic programs to better focus their curriculum.

Practical contributions of this research are twofold. Education providers, can draw upon these findings a better understanding of their students, students in the tourism orientation do care about tradition, still they do consider planning relevant, therefore this aspect should be relevant for the curriculum. This scale had never been studied in a Mexican population, as the two dimensions were verified, this gives support to Bearden *et al.* (2006) scale. Allowing for its use as a psychometric scale.

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First report about ants associated with *Diaphorina citri* Kuwayama in Mexican lime (*Citrus aurantiifolia* Swingle) in the Apatzingán Valley, Michoacán, México

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ABSTRACT

Objective: To carry out taxonomic studies that determine which formicine species are associated with the cultivation of Mexican lime.

Design/Methodology/Approach: The study was conducted in eight municipalities and 59 localities of the Apatzingán Valley region, Michoacán, México, where Mexican lime (*Citrus aurantiifolia* Swingle) is produced. A randomized complete blocks design was used in the experiment, where each municipality represented a block. The repetitions were the number of collections, while the experimental unit was a lime sprout infested with *Diaphorina citri* and ants. The collections were direct and were made with an entomological aspirator. The values obtained were analyzed with the SAS University Edition software (2018).

Results: The studied ants belong to the Dolichoderinae, Formicinae, Myrmicinae, Pseudomyrmecinae, and Ponerinae subfamilies, which represent 15 genera and the same number of species. The subfamilies with the greatest presence in the eight municipalities were: Myrmicinae, Dolichoderinae, and Formicinae. The species with the highest impact were *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*, while *Cardiocondyla minutior* and *Odontomachus* sp. had a lower impact.

Study limitations/implications: Social insecurity in the eight municipalities and the new form of coexistence.

Findings/Conclusions: The studied species prefer dry, warm, and disturbed sites.

Keyword: Myrmicinae, Dolichoderinae, *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*.

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INTRODUCTION

Ants are the most successful group in terrestrial environments; its almost 13,000 species present great diversity, both taxonomically and functionally. In México, 93 genera of ants, divided into 927 species, have been reported (Vásquez-Bolaños, 2015); however, they are a little explored group, despite their great success and diversity. They perform important predatory, herbivore, or detritivore functions and take part in the physicochemical processes of the soil, as well as in the decomposition and recycling of nutrients (Rojas, 2001). However, despite their importance in ecosystems, some species are considered economically important, since they can act as agricultural pests or be associated with other pests (Della, 2003). The *Solenopsis xyloni*, *Linepithema humile*, *Formica aerata*, and *Tapinoma sessile* ants impact the natural enemies of hemiptera (DeBach *et al.*, 1951; Martínez-Ferrer *et al.*, 2003; Bradford and Silverman, 2010). Another case is the Asian Citrus Psyllid *Diaphorina citri* Kirkaldi, which is associated with various species of ants such as *Pheidole megacephala*, *Brachymyrmex patagonicus*, *Solenopsis invicta*, and *Brachymyrmex obscurior* (Navarrete *et al.*, 2013). This association poses a serious threat to citrus farming, not only because the psyllid extracts the sap of the citrus plant, distorts the leaf, and causes sooty mold, but also because it transmits the *Candidatus liberibacter* bacterium, the causative agent of the worst disease that affects citrus in the world: Huanglongbing (HLB) or Citrus Greening (Halbert and Manjunath, 2004). The ecological interaction between *Diaphorina citri* and ants is important, because they protect the hemiptera from their natural enemies and quickly eliminate honeydew excretions, reducing bacterial and fungal infections; in return, ants obtain the honeydew from the plant that serves as an important food resource (Stachowicz, 2001).

Few current works refer to the psyllid-ants interaction. Navarrete *et al.* (2013) carried out one of the few of such studies in the state of Florida, USA. They mention that both in *Murraya paniculata* and in *Citrus latifolia*, parasitism of *Diaphorina citri* by *Tamarixa radiata* increased by 20.36% when ants were absent, compared with 0.39% parasitism when ants were present. Despite their importance, no studies about the ants associated with *Diaphorina citri* have been carried out in México. The symbiosis between *Diaphorina citri* and ants poses as threat to the important production of citrus in the state of Michoacán. Consequently, taxonomic studies were carried out to find out which ant species are associated with *Diaphorina citri* in the Mexican lime cultivation in the Apatzingán Valley, Michoacán, México.

MATERIALS AND METHODS

This study was carried out in the Apatzingán Valley region, which is located in the southwestern part of the state of Michoacán, México, and has a total area of 951,769 hectares (19° 05’ 03” N and 102° 21’ 15” W) (Google-Earth, 2022).

In the described region, a randomized complete block design was established, which enabled a comparison of the diversity of ants collected in each locality and municipality. Two formicines samplings were carried out. The first sampling was made from August 18 to 20, 2014, and 36 collections were obtained. The second sampling took place from March 16 to 24, 2015, with the same number of collections. A total of 72 collections were therefore gathered in eight municipalities: Aguililla, Apatzingán, Buena Vista, Gabriel Zamora, La Huacana, Francisco J. Múgica, Parácuaro, and Tepalcatepec. A population of 59 localities where Mexican lime (*Citrus aurantiifolia* Swingle) is produced was chosen from these municipalities.

In the orchards, the collection of ants was random. An entomological aspirator was used to isolate most of the specimens that were found in at least five sprouts of Mexican lime trees infested by formicines and by *D. citri* (Table 1).

The collected ants were deposited in Eppendorf® tubes containing 70% alcohol. Subsequently, they were classified by morphospecies and placed in glass jars with rubber seal lids. Some samples were mounted on No. 2 entomological pins and small ants were glued to cardboard triangles with Resistol 850® glue. Once the insects were labeled, a database was developed with Windows® Excel. The study was carried out at the Laboratorio de Invertebrados of the Facultad de Biología of the Universidad Michoacana de San Nicolás de Hidalgo (UMSNH), Morelia, Michoacán. A LEICA® stereoscopic microscope, the dichotomous keys developed by Mackay and Mackay (1989), and the descriptions proposed by Bolton (1994) were used to determine the genus of the ants. Finally, the specialist Mr. Miguel Bolaños Vásquez (MSc), of the Universidad Autónoma de Guadalajara, was consulted to determine the species of some ants. The SAS University Edition 2018 software was used to analyze the data on the number of ants associated with *Diaphorina citri*, with a Tukey test at 0.05 (Castillo, 2003).

RESULTS AND DISCUSSION

Field work

We collected 2,218 ants associated with *Diaphorina citri* in 59 locations belonging to eight municipalities in the state of Michoacán, México. The number of specimens collected ranged from 2 to 289 ants. Based on the highest to lowest number of ants collected, the municipalities where most ants were collected were: Buenavista, Apatzingán, F. J. Múgica, La Huacana, Parácuaro, Tepalcatepec, Aguililla, and Gabriel Zamora (Table 1).

The foregoing assumes that the density of ants is influenced by the surface of cultivated Mexican lime. Although this fruit is grown in 27 municipalities, most of the surface is concentrated in Buenavista (33%) and Apatzingán (32.5%); in the rest of municipalities the cultivated area is smaller, ranging from 3,500 and 160 to 1.6 hectares (SAGARPA, 2014).

Table 1 shows a contrast in the number of ants collected in the adjoining localities of Ampliación Presa del Rosario and Ejido Presa del Rosario, in the municipality of Apatzingán. Hypothetically, this is the result of the phytosanitary management that is given to the crop and the use of the land, since the lime trees in Ampliación Presa del Rosario are found in backyards and have little commercial area. In general, based on the ants-D. *citri* association, ants obtain sugary substances through associations with Hemiptera: Sternorrhyncha insects (Ramos and Serna, 2004). This relationship is called trophobiosis and is understood as the symbiotic association between organisms (Hölldobler and Wilson, 1990).

In México, research has already been carried out about the relationships between ants, insects of the Hemiptera order, and plants (Ibarra and Dirzo, 1990; Rico *et al*., 1998;

Table 1. Municipalities and localities of the Apatzingán Valley, Michoacán where ants associated with *Diaphorina citri* were collected (2014-2015).

Municipality	Locality	Number of ants collected (n)
Aguililla	El Aguaje	147
Apatzingán	Altamira	15
	Amp. Presa del Rosario	6
	Chiquihuitillo	18
	Ejido Apatzingán	39
	Ejido Presa del Rosario	97
	El Guayabo	9
	EL Mirador	45
	El Morado	6
	El Pino	22
	El Recreo	91
	La Nopalera	6
	Las Colonias	13
	Las Tinajas	10
	Mata de plátano	4
	Puerta de Alambre	12
	San Antonio la Labor	11
	San Fernando	18
	San Juan de los Plátanos	19
Buena Vista	18 de Marzo	109
	Catalinas	50
	Doroteo Arango	9
	El Porvenir	15
	El Recreo	31
	El Terreno	34
	Emiliano Zapata	145
	Felipe Carrillo Puerto	129
	Punta de Agua	56
	San José Piedras Blancas	2
	Santa Ana Amatlán	8
Gabriel Zamora	El Capire	140
La Huacana	Cupuán del Río	34
	Cupuancillo	24
	El Chauz	65
	La Peña	12
	Los Olivos	9
	Mártires de la parota	109
	Zicuirán	10
Francisco J. Múgica	El Ceñidor	289
	Nueva Italia	3

Table 1. Continues.

Municipality	Locality	Number of ants collected (n)
Parácuaro	Antúnez	58
	Buenos Aires	38
	Cancita	14
	Ciudad Morelos	12
	El Carrizo	14
	El Junco	17
	El Varal	8
	Úspero	16
Tepalcatepec	Catarino Torres	7
	Colomotitán	4
	El Montoso	4
	La Bocanada	83
	La Ordeñita	14
	La Romera	29
	Las Primaveras	3
	Los Huiranches	7
	Los Tambores	5
	Nuevo Corongos	6
	Pancha López	8

Torres *et al.*, 2000). The following ant-plant relationships stand out: myrmecophile, which only refers to those beneficial to the plant (Bentley, 1976); myrmecotrophy, in which the plant produces nutritive particles to feed the ants; myrmecophyte plants are those that have myrmecodomatia, morphological structures produced by the plant in which ants live (Gaume *et al.*, 2005); myrmecochory is the mechanism by means of which ants disperse seeds; and myrmecophily is the pollination carried out by ants (Hölldobler and Wilson, 1990). Nectaries are glands that produce sugary exudates which attract ants. They can be found in various parts of the plant, such as stems, leaves, and flowers. The appearance of nectaries could have been the result of a process of co-evolution between different species of plants and ants (Delabie *et al.*, 2003). Bentley (1976) and others indicate that there is a positive correlation between the frequency of plants with extra-floral nectaries and the abundance of ants in natural forests.

Taxonomic determination of the studied ants

The collected ants belong to five subfamilies, 15 genera, and the same number of species (Table 2). Vásquez-Bolaños (2015) mentions that 11 subfamilies, 93 genera, and 927 species are known in México, which is relevant because, in this work, five subfamilies were recorded in a relatively small region of the national surface. According to SIAP (2016), this fruit tree is grown in 24 states with an approximate area of 81,221.90 hectares. Therefore,

Table 2. Taxonomic grouping of ants associated with *D. citri* in Mexican lime cultivation, in the Apatzingán Valley, Michoacán, Mexico (2014-2015).

Locality	**Taxonomic categories**		
	Subfamily	**Genus**	**Species**
Aguililla	Dolichoderinae	*Dorymyrmex*	*flavus*‡
	Dolichoderinae	*Forelius*	*damiani*†
	Myrmicinae	*Monomorium*	*minimum*†
	Myrmicinae	*Novomessor*	*ensifer*†
	Formicinae	*Paratrechina*	*longicornis*†
Apatzingán	Myrmicinae	*Pheidole*	sp. ‡
	Myrmicinae	*Atta*	*mexicana*‡
	Myrmicinae	*Solenopsis*	*geminata*‡
	Myrmicinae	*Crematogaster*	*crinosa*‡
	Dolichoderinae	*Forelius*	*damiani*‡
	Dolichoderinae	*Dorymyrmex*	*flavus*‡
	Formicinae	*Paratechina*	*longicornis*†
Buena Vista	Myrmicinae	*Crematogaster*	sp. ‡
	Myrmicinae	*Monomorium*	*minimum*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Formicinae	*Paratrechina*	*longicornis*†
	Formicinae	*Nylanderia*	*terricola*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
Tepalcatepec	Myrmicinae	*Cardiocondyla*	*minutior*‡
	Myrmicinae	*Atta*	*mexicana*‡
	Myrmicinae	*Novomessor*	*ensifer*†
	Myrmicinae	*Monomorium*	*minimum*†
	Myrmicinae	*Crematogaster*	sp. †
	Dolichoderinae	*Dorymyrmex*	*flavus*‡
	Dolichoderinae	*Forelius*	*mccooki*†
	Formicinae	*Nylanderia*	*terricola*‡
	Formicinae	*Brachymyrmex*	*obcurior*†
	Formicinae	*Paratrechina*	*longicornis*†
	Pseudomyrmecinae	*Pseudomyrmex*	*elongatus*†
Gabriel Zamora	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Solenopsis*	sp. †
	Dolichoderinae	*Forelius*	*mccooki*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†

Table 2. Continues.

Locality	Taxonomic categories		
	Subfamily	Genus	Species
La Huacana	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Novomessor*	*ensifer*†
	Myrmicinae	*Monomorium*	*minimum*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†
Francisco J. Múgica	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Solenopsis*	sp. †
	Myrmicinae	*Crematogaster*	sp. †
	Dolichoderinae	*Azteca*	sp. †
	Dolichoderinae	*Forelius*	*mccooki*†
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†
	Formicinae	*Brachymyrmex*	*obcurior*†
	Ponerinae	*Odontomachus*	sp.
Parácuaro	Myrmicinae	*Atta*	*mexicana*†
	Myrmicinae	*Solenopsis*	sp.†
	Myrmicinae	*Crematogaster*	sp.†
	Dolichoderinae	*Forelius*	*mccooki*†
	Dolichoderinae	*Forelius*	*pruinosus*†
	Dolichoderinae	*Azteca*	sp. †
	Pseudomyrmecinae	*Pseudomyrmex*	*simplex*†
	Formicinae	*Paratrechina*	*longicornis*†

‡=taxonimical determination of the ants carried out by Miguel Vásquez-Bolaños.
†=taxonimical determination of the ants carried out by Ana Leticia Escalante Jiménez.

the presence of formicines can be considered as a bioindicator for the phytosanitary management of different citrus agroecosystems since some are more disturbed than others.

The subfamilies with the greatest presence in the eight municipalities were: Myrmicinae, Dolichoderinae, and Formicinae. These results match the findings of Bolton (1995), who points out that only six of the 16 registered subfamilies of the ant fauna in question are distributed in all of the eight zoogeographical regions: Cerapachyinae, Dolichoderinae, Formicinae, Myrmicinae, Ponerinae, and Pseudomyrmecinae.

Khachonpisitsa *et al.* (2020) and other authors confirm that the most abundant subfamilies are Mirmicinae which comprises 36.70% of all genera and 40.83% of all species, followed by Formicinae (17.43% and 10.59%), Dorylinae (11.01% and 9.83%), and Dolichoderinae (6.42% and 6.62%).

In the Apatzingán Valley, the subfamily with the least presence was Ponerinae, which was only collected in the municipality of F.J. Mugica. For their part, two to five species of the Myrmicinae subfamily, two to three Dolichoderinae species, and one to three Formicinae species were found in the eight municipalities (Figure 1). Rojas (2011) mentions that the ant fauna of the soil comprises 407 species: Myrmicinae is the most diverse subfamily, it includes 53% of the total number of species, and it dominates the tropical areas of México, while Formicinae has been collected in temperate zones. The Dolichoderina subfamily has a cosmopolitan distribution and can be found everywhere, except for the polar areas (Ward *et al.*, 2010). It comprises approximately 900 described species (Bolton *et al.*, 2007), including some of the world's most successful invaders, such as the Argentine ant (*Linepithema humile*), the ghost ant (*Tapinoma melanocephalum*) and the white-footed ant (*Technomyrmex* spp.) (Williams 1994; Holway *et al.*, 2002). The adaptation and success of the ants is caused by the presence of the metapleural gland, whose phenylacetic acid secretion differentially inhibits the growth of microorganisms within the nests (Maschwitz *et al.*, 1970).

Regarding the distribution of ant genera and species by municipality, the subfamily Formicinae: *Paratrechina longicornis* was collected in all the study sites, followed by Myrmicinae: *Atta mexicana* (6 municipalities). In contrast, *Solenopsis geminata*, *Cardiocondyla minutior*, *Pheidole* sp., and *Crematogaster crinosa* were only recorded in one municipality. For its part, Pseudomyrmecinae: *Pseudomyrmex simplex* was found in five municipalities; meanwhile, two species from this subfamily (*Pseudomyrmex elongatus* and Ponerinae: *Odontomachus* sp.) were collected only in one municipality. (Table 3).

Formicinae: *Paratrechina longicornis* is present in all municipalities (Table 3). This species is defined as polygynous and polycalic; it dominates in highly stressed or disturbed environments, and limits the existence of other species (Smith, 1965). It should be monitored, given its omnivorous, opportunistic, predatory, and scavenger status, and its adaptability to both dry and humid environments. It is ecologically important —because it can displace other native ant or invertebrate species—, economically important —as an agricultural pest—, and socially important —because it causes damage to domestic spaces.

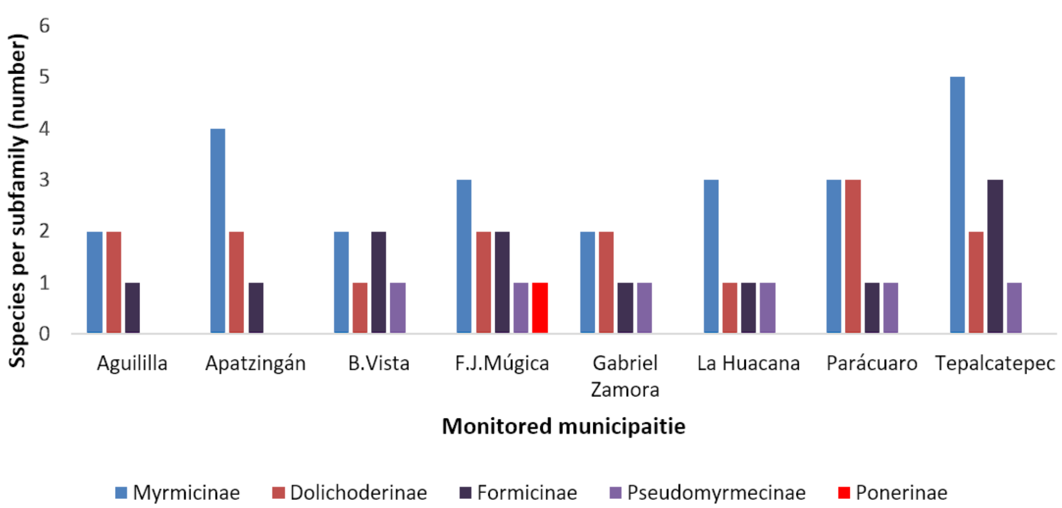

Figure 1. Ant subfamilies collected in eight Apatzingán Valley municipalities, Michoacán, Mexico (2014-2015).

Table 3. Distribution of ants associated with *Diaphorina citri* collected in Mexican lime trees, in eight municipalities of the state of Michoacán, Mexico (2014 and 2015).

Species \ Municipality	Aguililla	Apatzingán	Buena Vista	Francisco J. Múgica	Gabriel Zamora	La Huacana	Tepalcatepec	Parácuaro
Atta mexicana	Presente	Ausente	Ausente	Presente	Presente	Presente	Presente	Presente
Azteca sp.	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Ausente
Brachymyrmex obcurior	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Presente	Ausente
Cardiocondyla minutior	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Crematogaster crinosa	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Crematogaster sp.	Ausente	Ausente	Presente	Presente	Ausente	Ausente	Presente	Presente
Dorymyrmex flavus	Presente	Presente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Forelius damiani	Presente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Forelius mccooki	Ausente	Ausente	Ausente	Presente	Presente	Ausente	Presente	Presente
Forelius pruinosus	Ausente	Ausente	Presente	Ausente	Presente	Presente	Ausente	Presente
Monomorium minimum	Presente	Ausente	Presente	Ausente	Ausente	Presente	Presente	Ausente
Novomessor ensifer	Presente	Ausente	Ausente	Ausente	Ausente	Presente	Presente	Ausente
Nylanderia terricola	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Presente	Ausente
Odontomachus sp.	Ausente	Ausente	Ausente	Presente	Ausente	Ausente	Ausente	Ausente
Paratrechina longicornis	Presente	Presente	Presente	Presente	Presente	Presente	Presente	Presente
Pheidole sp.	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Pseudomyrmex simplex	Ausente	Ausente	Presente	Presente	Presente	Presente	Ausente	Presente
Pseudomyrmex elongatus	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente	Presente	Ausente
Solenopsis geminata	Ausente	Presente	Ausente	Ausente	Ausente	Ausente	Ausente	Ausente
Solenopsis sp.	Ausente	Ausente	Ausente	Presente	Presente	Ausente	Ausente	Presente

(green) = Ausente. (red) = Presente.

Vásquez-Bolaños (2015) reports that *P. longicornis* is present in various states of the Mexican Republic, but not in Michoacán; therefore, this is the first time that this species has been reported in the state.

Its dispersion is favored by trade and, in general, by human transport. It currently is one of the wandering ant species with the widest geographic distribution (Wetterer *et al.*, 1999). Regarding the species Myrmicinae: *Atta Mexicana*, Rojas (1989) reports its presence in several Mexican states, but not in Michoacán. This species is cataloged as a pest of economic importance, because it causes considerable losses when it defoliates timber trees and agricultural crops (Serratos *et al.*, 2017). Infante-Rodríguez *et al.* (2020) point out that the leaves that are not attacked have alkaloids, phenols, flavonoids, and tannins, as well as high concentrations of chlorogenic acid, (-)-epicatechin, quercetin-3, 4-di-O-glucoside, shikimic acid, ellagic acid, and scopoletin. These compounds have been linked to antifungal effects and deter leafcutter ants.

Despite the chemical defense strategies of the plants —which include the production of specific secondary metabolites, which are toxic to leafcutter ants (Cherrett 1972; Boulogne *et al.*, 2012; van Bael *et al.*, 2011), or which also inhibit the growth and development of their symbiotic fungus, *Leucoagaricus gongylophorus* (Möller) (Miyashira *et al.*, 2012; Lobo-Echeverri *et al.*, 2016)—, it has been reported that the symbiotic fungus of some *Atta* species can detoxify certain phenolic compounds present in the leaves of forage plants (Cherret 1980; Powell and Stradling, 1986; Nichols-Orians, 1991). In addition, the worker ant obtains certain enzymes (laccases or polyphenol oxidases) from the fungus. These fungal enzymes pass through the ant gut without becoming denatured (De Fine Licht *et al.*, 2013), are added by the ants as fecal droplets to processed plant material (Rockwood 1976), and help to detoxify some compounds present in the leaves (Aylward *et al.*, 2013). Consequently, the range of plants that leafcutter ants can harvest seems to be broadened through fungal-derived enzymatic detoxification techniques. The presence of this ant in the eight municipalities studied is worrisome, because in view of the lack of a comprehensive lime crop management, various cultural tasks —such as gravity irrigations, pruning, and nitrogen-based fertilization— induce the permanent presence of sprouts, propitiating the ideal environment for colonization by ants of the genus *Atta mexicana*.

As for *Monomorium minimum*, the little black ant is an occasional invader of human dwellings (Smith 1965). They are diurnal and consume immobilized arthropods, as well as sternorrhyncha honeydew (Stein and Thorvilson, 1989). *M. minimum* was found in soybean aphid (*Aphis glycinas*) populations, harassing or killing *Orius insidiosus* and *Harmonia axyridis*, which resulted in reduced predation and an up to 10-fold increase in aphid numbers when ants were present. Ants were not observed directly interfering with the *Aphidius colemani* parasitoid, although the number of parasitized aphids was higher in aphid colonies left unattended by ants (Sharaf *et al.*, 2015).

According to its biology and behavior when it becomes associated with *D. citri*, this situation results from the fact that this is where *M. minimum* finds its ideal food diet (meat=proteins and sugars=energy source). Therefore, the contribution of this work was the accurate identification of the formicines, which enabled an easier and more effective ant control. An erroneous identification can lead to inappropriate and expensive (time and money) control tactics and lead to unnecessary risks to people or the environment.

For its part, the genus *Crematogaster* is a widespread and distinctive lineage of myrmicine ants (Hölldobler and Wilson 1990). In most species, colonies can be huge (covering the canopy of trees) or tiny (contained within a single dead twig). Huge colonies are often polydomous, aggressive, and territorial. *Crematogaster* appear to be very generalized collectors and omnivores, with most of its species nesting on dead wood and in stem, branch, or trunk hollows (Longino, 2003).

The genus *Crematogaster* is clearly monophyletic, with a unique and apomorphic arrangement of postpetiole and gaster. The teardrop-shaped gaster points backwards and the postpetiole is attached to the dorsal surface of the fourth abdominal tergite. Consequently, the gaster is suspended below the postpetiole, rather than being clearly behind it. The petiole has no dorsal node and, when the gaster is elevated, the petiole fits level with the propodeum. This combination of characteristics is probably related to the defensive or offensive behavior during which the workers wave their gasters in the air, exuding a drop of venom on the spatula of the stinger (Buren, 1959).

The antennae have 11 segments with the exception of a lineage of Asian and African species, which have antennae with 10 segments and a terminal bundle of 2-4 segments. The propodeum usually has a pair of dorsal spines (lack of spines is rare among New World species). Species differ in the petiole and postpetiole shape, general pilosity characteristics, and surface sculpture (Bolton, 1995).

In this sense, *Crematogaster crinosa* was reported in Costa Rica as a very widespread species associated with lime cultivation, that prefers isolated and seasonally dry habitats. Workers are omnivores, are attracted to protein and carbohydrate baits, feed on dead or injured insects, visit extrafloral nectaries, and take care of homoptera. When their nests are disturbed, they can be aggressive. *Crematogaster crinosa* is the only member of the group that regularly dominates mangrove habitats (Longino, 2003). The presence of this ant in the Apatzingán Valley, Michoacán, is a clear sign that insects maintain magnificent alarm sensors, which makes them excellent bioindicators of an ecosystem's environmental health status; being poikilotherms, they easily respond to any stimulus, through physiological or biochemical mechanisms. In other words, the presence of this species suggests that most of the lime orchards in the study sites are neglected and old.

The subfamily Pseudomyrmecinae is monophyletic; it arose during the Cretaceous and probably most of its diversification took place during the Tertiary (Wilson and Hölldobler, 2005). They are fast-moving ants, with large eyes and a well-developed stinger; they generally inhabit the tree layer (Ward, 1990). Larsen and Philpott (2010) mention that *Pseudomyrmex ejectus*, *Pseudomyrmex simplex*, and *Pseudomyrmex* PSW-53 nest in twigs and act as predators of the coffee berry borer. Their presence may not be as closely related to the intensity of shade management as has been suggested for other arthropod predators of the coffee berry borer. The *Pseudomyrme simplex* species has been registered in the state of Tamaulipas, México (Coronado-Blanco, 2013).

Ants have a remarkable functional diversity, since they comprise a wide spectrum of trophic guilds. However, from the point of view of their diet, ants always select the most nutritious materials (Stradling, 1978). They take food from various trophic levels such as seeds, nectar, fungi, insect secretions, carcasses, faeces, live prey (various arthropods), or a combination of these elements (Bolton, 1994; Petal, 1978).

In addition, *Citrus aurantiifolia* is a citrus species that produces vegetative sprout throughout the year. Consequently, tender tissues are available that favor the presence of overlapping generations of *D. citri* and allows nymphs to feed on the sap of leaves and tender stems, during their five stages. The result of this food process is the secretion of large amounts of sugar that largely define the Citrus-*Diaphorina*-formicines association.

The genera *Azteca* and *Forelius* —part of the subfamilies and genera Myrmicinae: *Solenopsis* and *Dolichoderinae*— are commonly found in warm environments and arid zones; according to the climatic classification developed by García (1987), the Apatzingán Valley is a region with an arid climate and scarce rainfall.

Finally, the records presented by Soto-Cárdenas *et al.* (2019) also agree that the most abundant subfamily was Myrmicinae, followed by Dolichoderinae and Formicinae, while the least abundant was Ponerinae. The least abundant genera were: *Brachymyrmex*, *Odontomachus*, and *Cardiocondyla*.

Statistical analysis of ants by municipality, genera, and species

The information from 72 collections was statistically analyzed; the collections were separated in eight blocks —one block per each of the municipalities severely infested by *D. citri*. The results show that the experimental model was significant, given the value of F_{cal}=2.19 *vs.* Pr>F=0.0019. On the contrary, the blocks were not significant when F_{cal}=1.20 *vs.* Pr>F=0.3216, which indicates that the municipalities are physically similar (climate, altitude, variety of plantations, and agronomic management). Likewise, ant dispersion can be favored by the lack of phytosanitary control or good practices when the materials used during the sowing of the orchards and the harvesting of the limes are handled, as well as during the transportation of plants to other plots or the packaging of the fruits.

The result obtained from the analysis of the dependent ant variable was significant. This value (F_{cal}=3.34 *vs.* Pr>F=0.0006) means that there is a very close ants-*D. citri* relationship and that at least one genus of ants represented greater association. Therefore, according to the decision rule, the null hypothesis is rejected (Table 4). The determination coefficient (r^2) explains by 86.30% that the density of ants is closely related to *Diaphorina citri*. The result of the coefficient of variation (CV=28.45) suggests that the accuracy with which the treatments were compared is reliable and that the experiment is statistically acceptable (Table 4).

For the interpretation of Tukey's test (α=0.05), the assigned letters belonged to five groups. However, the most abundant genera and species in this research were *Paratrechina longicornis*, followed by *Forelius mccoki*, and *Atta Mexicana*: statistically, they presented the highest mean and, during the field practice, they showed greater density in the municipalities studied. These results reflect a close association with *D. citri* and, in the case of *Atta mexicana*, it is related to vegetative sprouting. The genera with the least impact were *Odontomachus* sp. and *Cardiocondyla minutior* (Table 5).

Table 4. Analysis of variance of the number of ants associated with *Diaphorina citri* in Mexican lime orchards, in the Apatzingán Valley, Michoacán (2014-2015).

Source of Variation	Degrees of Freedom	Sum of Squares	F_{cal}	Pr>F
Modelo	23	202221.73	2.69	0.0019
Error	48	156937.55	-0-	-0-
Total	71	359159.30	-0-	-0-
Blocks	7	25472.19	1.10	0.3706
Ants	14	174783.15	3.34	0.0006

R^2	Coefficient of variation
0.8630	28.4532

Table 5. Grouping of ants associated with *Diaphorina citri* in Mexican lime orchards, in the Apatzingán Valley, Michoacán (2014-2015).

Tukey group	Average	Ant
A	31.50	*Paratrechina longicornis* (Latreille, 1802).
A	30.50	*Forelius mccooki* (McCook 1880).
A	30.00	*Atta mexicana* (F. Smith, 1858).
A	30.00	*Forelius pruinosus* (Roger, 1863).
B	23.250	*Forelius damiani* Guerrero & Fernández, 2008
B	22.71	*Crematogaster crinosa* Mayr, 1862
B	22.50	*Crematogaster* sp. Lund 1831.
C	19.75	*Monomorium mínimum* (Buckley, 1867).
C	16.37	*Pseudomyrmex simplex* (Smith, 1877).
C	13.00	*Dorymyrmex flavus* (McCook, 1879).
C	11.25	*Solenopsis* sp. (Westwood, 1840).
D	7.50	*Pseudomyrmex elongatus* (Mayr, 1870).
D	7.50	*Azteca* sp. Forel, 1878.
D	7.00	*Brachymyrmex obscurior* (Forel, 1893).
D	7.00	*Nylanderia terrícola* (Buckley, 1866).
D	7.00	*Novomessor ensifer* (Forel, 1899).
D	6.75	*Pheidole* sp. (Westwood, 1839).
D	6.75	*Solenopsis geminata* (Fabricius, 1804).
E	1.00	*Cardiocondyla minutior* Forel, 1899.
E	1.00	*Odontomachus* sp. Latreille, 1804.

Means with the same letters are not significantly different. Means with different letters are statistically different.

CONCLUSIONS

The municipalities where more ants were collected were Buenavista, Apatzingán, and F. J. Múgica. In the 59 localities, five subfamilies associated with lime cultivation and with *Diaphorina citri* were collected: Dolichoderinae, Myrmicinae, Formicinae, Pseudomyrmecinae, and Ponerinae. The dominant species were *Paratrechina longicornis*, *Forelius mccooki*, and *Atta mexicana*. The least abundant species were *Solenopsis geminata*, *Crematogaster crinosa*, *Cardiocondyla minutior*, and *Odontomachus* sp. The genera Azteca, *Forelius*, *Dorimyrmex*, and *Paratrechina* were collected in the driest, warmest, and most disturbed municipalities. *Diaphorina citri* and the ants that cohabit in the Apatzingán Valley region are adapted to a warm climate without a defined winter. Lime plants grow sprouts on a permanent basis, a condition that favors the plant-insect relationship. The association between *Diaphorina citri* and ants from the following genera was confirmed: *Pheidole*, *Brachymyrmex*, and *Solenopsis*.

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AGRO
PRODUCTIVIDAD