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Conclusiones: Son la generalización de los resultados obtenidos; deben ser puntuales, claras y concisas, y no deben llevar discusión, haciendo hincapié en los aspectos nuevos e importantes de los resultados obtenidos y que establezcan los parámetros finales de lo observado en el estudio.
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Nanoparticle sterilization methods for biomedical applications in animals

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ABSTRACT
Objective: To review different nanoparticle sterilization methods for their use in biomedical applications in animals.
Approach: Sterilization is used to obtain a microorganism-free product without altering its physicochemical characteristics during its preparation, storage, or administration route. This review explores different sterilization methods and their advantages and disadvantages on the nanoparticle level.
Study limitations/implications: Nanoparticles are used in animal production, including their parenteral administration. Therefore, establishing the characteristics of different technologies applied to sterilize nanoparticles is essential to ensure the delivery of sterile products preventing health risks.
Conclusions: The use of nanotechnology in livestock production offers several advantages for animal nutrition, reproduction, and health, among other things. When nanoparticles must be sterilized, choosing the most suitable method is essential. This depends on the amount of product and its compound type because each technique has specific requirements that must be taken into account to be ready for potential changes in the structure and availability of the final product.

Keywords: Animals, Parenteral application, Nanoparticles, Sterilization.

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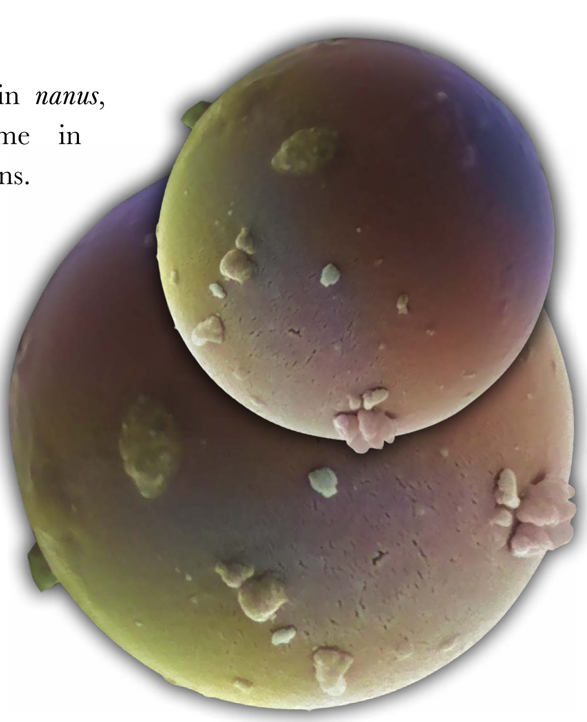
INTRODUCTION

The word "nano" comes from the Latin *nanus*, which means dwarf. Nanoparticles come in different sizes and react in different conditions. They can measure between 1 and 100 nanometers. Nanoparticles are divided into organic, inorganic, emulsions, and nanoclays, depending on their chemical composition. Nanoparticles have been used in animal production to provide minerals, vitamins, proteins, and other essential nutrients (Bunglavan *et al.*, 2014). They are also used to improve intestinal health, regulate gut microbiota, maintain blood homeostasis, increase immune response, and improve semen



quality. Nanoparticles have been employed as dietary supplements since they cross the digestive tract membranes more easily than larger molecules. They also increase the bioavailability and cell uptake of minerals and reduce mineral antagonism (Abdelnour *et al.*, 2021). Nanoparticles can also be used to diagnose and treat illnesses, administer medication, and apply other compounds by parenteral route (Khin *et al.*, 2012), in which case they must be sterilized in order to avoid endangering the animal's health.

The sterility of a product is the absence of any living microorganism that could endanger health. There are different methods to sterilize nanoparticles, such as sterile filtration, autoclave, ionizing (gamma irradiation) and non-ionizing radiation (ultraviolet, infrared, and microwave irradiation), alongside others such as sterilization by hydrogen peroxide plasma, ethylene oxide, and formaldehyde (Bernal *et al.*, 2021).

Nowadays, sterility assurance levels (SALs) of 10-6 are accepted: a probability of no more than one viable microorganism in a million molecules of the sterilized product (von Woedtke and Krammer, 2008).

In addition, the morphology, the physicochemical properties, and the biological performance of the product must not alter after the sterilization method is applied — regardless of the nature of the said method (Fracalossi *et al.*, 2016). Meanwhile, preparation, packaging, and storage conditions must be adequate to prevent contamination and maintain the final product›s sterilization efficiency (McKeen, 2018).

STERILIZATION METHODS

Sterile filtration

Using 0.2-0.45 μm membrane filters, this technique removes microorganisms found in liquid nanoformulations. This procedure can be useful to sterilize thermosensitive and chemical-sensitive nanosystems. However, it has limitations related to viscosity and filtration, and it can even alter the integrity of the nanoformulation. For this reason, its use should be evaluated for each particular case (Bernal, 2021).

Heat sterilization (autoclave)

Among the available sterilization methods, the steam method is one of the most widely used due to its low cost and non-toxicity (Adler *et al.*, 1998). The steam sterilization method or autoclave removes polluting agents with high-pressure steam, at a minimum temperature of 120 °C, for approximately 15 to 20 minutes, depending on the size and quantity of the material to be sterilized. Sterilization is achieved when the entire surface reaches a temperature of 121 °C (Block, 2001; Vetten *et al.*, 2014). To ensure the method's reliability, the temperature, time, and air entrance into the equipment must be controlled (Lerouge, 2010). This method can be used in metal nanoparticles, although the particles may slightly increase in size and crystallize due to the temperature of the autoclave. Nonetheless, steam sterilization has proved to be adequate for mineral nanoparticles, which do not present changes in size or morphology (Santos *et al.*, 2012).

This method can be applied to non-aqueous liquids or dry powder materials. It is not recommended for temperature-sensitive materials (Fracalossi *et al.*, 2016). It destroys or inactivates all microorganisms that are exposed to steam (Stammen *et al.*, 2012). The temperature may vary between 120 and 170 °C, with a 20 to 150-minute exposure (Fracalossi *et al*., 2016).

Nakamura *et al*. (2015) tested two sterilization methods (autoclave and gamma irradiation) to analyze the protein breakdown caused by the sterilization process. Since autoclave generated lower protein oxidation than sterilization by gamma irradiation, it was considered adequate for susceptible pharmaceutical and biopharmaceutical products.

Fesharaki *et al*. (2010) sterilized a *Klebsiella pneumoniae* culture containing selenium nanoparticles at 121 °C, 17 PSI, for 20 minutes. No chemical changes were found in the selenium nanoparticles during the sterilization process. When they sterilized diclofenac sodium in an autoclave at a temperature of 123 °C, 1.1 kg cm^{-2}, in a 25 to 30-minute range, Roy *et al*. (2001) observed impurities in the medication; the use of other sterilization methods was therefore recommended. When sterilizing chitosan hydrogel nanoparticles with gamma radiation at 8, 12, and 25 kGy, in an autoclave at 100, 110, and 121 °C, during 5, 10, 15, and 30 minutes, sterilization by autoclave melted chitosan particles. In contrast, with gamma radiation, the particles kept their original structure (Galante *et al*., 2016).

Sterilization with ionizing radiation (gamma irradiation)

Gamma irradiation has been proved to destroy nucleic acids, based on the fact that atoms are ionized and that the free electrons that are created interact with DNA or enzymes or cause the membranes to break, thus destroying microorganisms. This sterilization method uses a photon source (Craven, 2020). The usual sterilization dose is 25 kGy and maximum temperatures fluctuate between 30 and 40 °C. Irradiation usually requires $cobalt^{60}$ because matter absorbs it at a low rate per unit of time (120 kGy-s). Other isotopes, such as $cesium^{137}$ and $iridium^{192}$, are used for industrial and medical materials (Drobny, 2019).

This sterilization method is recommended for thermosensitive medicines and excipients (Domariska *et al*., 2020). However, irradiation on protein nanoparticles, especially in solution, produced reactive oxygen species capable of changing the protein's structure and inducing degradation effects and a general deterioration of their biological properties (Fazolin *et al*., 2020).

When materials with added antioxidants are sterilized, gamma sterilization may be detrimental (Yagoubi *et al*., 1997), besides producing unwanted radicals in polymers (Cottam *et al*., 2009).

However, gamma-ray sterilization has proved overall to be an effective method and is accepted by the European and US pharmacopeias (Hasanain *et al*., 2014). Some products currently sold in these countries —such as QuikClot®, which helps blood clots form quicker in open wounds— are aluminum silicate nanoparticles sterilized with gamma rays (QuikClot®, 2021).

Ultraviolet (UV) irradiation sterilization

Ultraviolet light emits photon energy. When a molecule absorbs this energy, it emits photons, which turn the molecule fluorescent and cause it to get excited. The energy is subsequently released as heat and returns to its natural state, producing biochemical changes in the molecule (Gray, 2013). The World Health Organization (WHO) establishes 100-400 nm wave ranges for ultraviolet radiation and divides them into three bands: UVA (315-400 nm), UBV (280-315 nm), and UVC (100-280 nm) (WHO, 2002).

UV irradiation is an adequate method for the sterilization of polymeric biomedical materials (Sionkowska *et al.*, 2006). Its bactericidal effect takes place at 240-280 nm and depends on the time of exposure. However, Chen *et al.* (2020) and others have used them at 365 nm.

Several studies have been carried where the large-scale application of UV-ray sterilization was used to eliminate spores of the genus Mycobacterium (Xu *et al.*, 2003). Air pollutants such as formaldehyde and volatile organic compounds (Liu *et al.*, 2019); bacteria such as *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, and *Staphylococcus aureus* (Green and Scarpino, 2001); as well as to inactivate airborne viruses (Tseng and Li, 2005) and to sterilize water (Cheng *et al.*, 2020; Timmermann *et al.*, 2015).

Ultraviolet radiation is inexpensive, easy to operate, and allows for quick sterilization. Nonetheless, its effectiveness depends on the sensitivity of bacteria to this radiation, which might be affected by factors such as the environmental pH and the bacterial growth phase. Finally, information on how this process affects nanomaterials in nanoparticles is scarce since many studies fail to mention either this situation or which wavelength they employed (Bernal *et al.*, 2021).

Sterilization with different gases

Some advantages of this sterilization method include its environmental safety and its lack of toxic residues. It is used in thermo- and humidity-sensitive compounds (Table 1), ensuring their microbicidal efficiency (Rutala and Weber, 2019).

Sterilization with ethylene oxide and formaldehyde

Ethylene oxide is used to sanitize medical devices. It is a strong alkaline agent that denatures the nucleic acids and functional proteins of microorganisms. For this reason, it is an excellent sanitizer, although it has the disadvantage of being flammable and explosive, in addition to producing toxic and carcinogenic residues. Formaldehyde is useful for sterilizing highly thermosensitive materials; its main limitations are its toxicity and carcinogenicity (Velten *et al.*, 2014).

SELECTING A STERILIZATION METHOD

As has been mentioned beforehand, each sterilization method has advantages and disadvantages. Therefore, several factors must be taken into consideration in order to decide which method to use: the physicochemical properties of the materials that constitute

Table 1. Different compounds used in the gas sterilization method Cycle time Temperature Product.

Gas type	Cycle time	Temperature (°C)	Product
Ethylene oxide	15 h	40	Medical instruments, sensitive to heat
Peroxide plasma	28 a 38 min	50	

Fracalossi *et al.* (2016).

the nanoparticle, the type of nanoparticle, the complexity of the involved materials, and their rate and physical state, as well as the susceptibility of the encapsulated compound. Sterilization by filtration is recommended for nanoparticles smaller than 200 nm, have low viscosity, and have a low percentage of solids. Radiation is better for other materials, such as those with a low melting point (Bernal *et al.*, 2021). The sterilization process might affect the nanoparticle's structure from the surface to the interior, which could cause it to lose its stability in an aqueous environment and many of its properties and characteristics. In addition, the ease with which each sterilization method is carried out and the availability of the necessary equipment are important factors to consider. However, except gamma radiation, all methods are easy to apply. Finally, terminal sterilization must also be taken into consideration. This type of sterilization refers to the process whereby a product is sterilized within its final packaging or container in order to ensure it is free of microorganisms, as well as of endotoxins and β–glucans (Neun and Debrovolskaia, 2019). This process is applied mainly to pharmaceutical products. However, terminal sterilization does not compensate for the lack of control and good practices in the previous sterilization process. The most commonly used methods include humid heat sterilization, irradiation, and the use of ethylene oxide. Sterilization methods require validation which involves biosafety, structural characterization, and toxicity tests (Bernal *et al.*, 2021).

CONCLUSIONS

Livestock production faces challenges when improving animal productivity and offering high-quality products without increasing consumer costs. Nanotechnology opens great possibilities in this domain, since it improves productive behavior and nutrient availability; moreover, it is employed in veterinary medicine. Therefore, when nanoparticles need to be sterilized, the method of choice will depend on the size, quantity, and type of product,

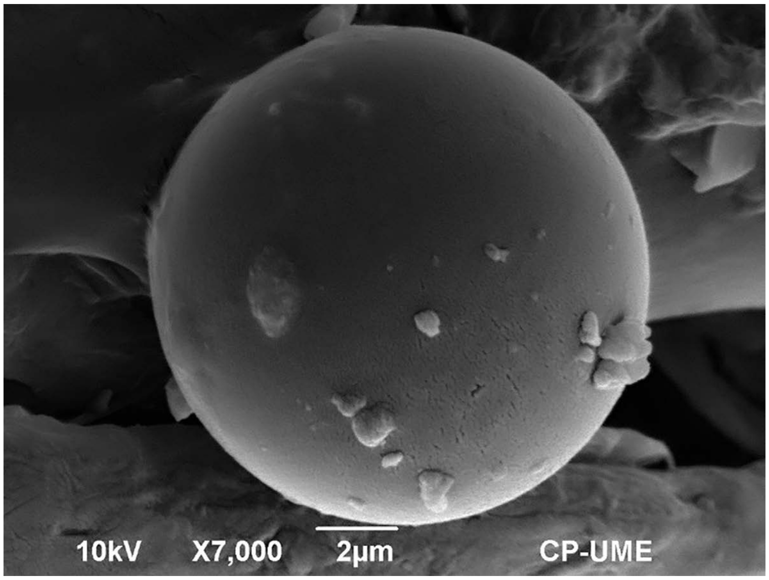


Figure 1. Nanosphere characterized by the Unidad de Microscopía Electrónica del Colegio de Postgraduados (CP-UME), Montecillo, Texcoco, Mexico.

since each method requires specific conditions, such as temperature and time of exposure, which can modify the physicochemical structure of the final product. The packaging type and material must also be considered because it might cause unwanted reactions if it is not sterilized. Hence the importance of terminal sterilization.

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Personal features and management activities related to cattle welfare in Loma Bonita, Oaxaca

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ABSTRACT

Objective: To describe the personal features, as well as the management activities related to cattle welfare in Loma Bonita, Oaxaca, Mexico.

Design/Methodology/Approach: This is a cross-sectional, descriptive, and quantitative study. The sampling was non-probabilistic for convenience. Between December 2019 and March 2020, a questionnaire was applied to 27 farmers.

Results: The average age of the ranchers is 44.6 years; 55.6% have more than 20 years of experience; 25.9% herd their cattle using a stick; 48.1% do not disinfect the udders; and 25.1% do not wash their hands during milking.

Study Limitations/Implications: The results and conclusions are limited to the sample used in the present study. Therefore, extending them to all livestock farmers in the municipality would require a broader study that considers statistical techniques of probabilistic sampling.

Findings/Conclusions: There are management activities outside the parameters established by the appropriate sanitary and welfare practices of cattle. However, the personal features indicate the existence of conditions related to the livestock activity in Mexico, that encourage participants to propose and carry out programs, plans, and strategies aimed at promoting and applying practices on animal welfare, thereby improving the productive, reproductive and profitability parameters.

Keywords: Cattle, personal features, animal welfare.

INTRODUCTION

The issue of cattle welfare acquired greater relevance, particularly in the first two decades of this century, given its association with the increase in productive and reproductive parameters, as well as economic benefits (Ramírez-Iglesia *et al.*, 2016). Additionally, consumers are concerned and exercise pressure regarding livestock breeding or the production of food of animal origin, of which they are the final consumers. Consequently, improving the conditions and traditional management enables ranching practices which include better treatment and attention to animals (García-Castro *et al.*, 2019).

Image by Elsemargriet at Pixabay

Despite the above, the study of activities related to animal welfare in Mexico does not constitute yet a priority objective in agricultural and livestock development plans. In fact, researches about indicators that make it possible to measure welfare in cattle herds —in which the characteristics of each region, municipality or locality are considered— remain scarce. Another problem that has been detected within the Mexican agricultural and livestock sector is that —even if technicians, researchers, government agencies, and others make suggestions about the adoption of technologies, techniques or methods aimed at increasing the livestock productivity— these suggestions have not been adopted or incorporated into the production process, because the personal features of the producers are not taken into account (Salas-González *et al.*, 2013).

In addition, not only does Mexico have a wide range of climates and geographic conditions, but also a cultural, social, and economic diversity that generates variations in idiosyncrasy, traditions, customs, manner, and treatment towards cattle, from one geographic space to another, or even within the same space. (Juárez-Barrientos *et al.*, 2015). Therefore, research on animal welfare is expected to have an impact in at least three aspects: a) to contribute with producers to improve livestock management conditions that will be reflected in higher productive, reproductive and financial parameters; b) to generate public policies for a more humane and comfortable animal production; and c) to guarantee that consumers can purchase higher quality products of animal origin, produced under welfare conditions.

In the case of Loma Bonita, Oaxaca, Mexico, some effort has been made to characterize cattle production units, both in this municipality and in surrounding towns. However, there is a lack of information regarding producers and management activities related to the welfare of the cattle, even though cattle ranching is one of the main economic activities of the area. The objective of this study was to describe the personal features of the producers, as well as the management activities related to cattle welfare in production units of the municipality of Loma Bonita, Oaxaca, Mexico.

MATERIALS AND METHODS

Study Area Location

The work was carried out in cattle production units of the municipality of Loma Bonita, Oaxaca, which is located between the following geographic coordinates: 95° 53' W and 18° 06' N; at 30 masl (INEGI, 2002). The climate is hot subhumid (Aw2(i')g), with a >1600 mm average annual precipitation, and 25 °C annual average temperature (García, 2004), with maximum temperatures exceeding 40 °C.

This is a cross-sectional, descriptive, and quantitative research (Cortés-Cortés and Iglesias-León, 2004). The population under study were dairy cattle producers. The reporting units were the owners and/or managers of the production unit.

The sampling was non-probabilistic for convenience, with the support of the person in charge of the Regional Ranchers' Association of Loma Bonita. Twenty-seven surveys were applied from December 2019 to March 2020. The questionnaire was composed of four personal factors: age (years), gender, experience in the activity, and schooling. According to the literature (Barba *et al.*, 2015; Pérez and Larios, 2018; Cuevas-Reyes, 2019; Flores

-González *et al.*, 2019), these factors can influence the adoption of technology and/or new knowledge by producers. The management activities related to cattle welfare included seven practices: 1) roundup, 2) colostrum supply, 3) navel cutting, tying, and disinfection 4) dehorning, 5) dehorning method, 6) hand washing, and 7) udder washing or cleaning. The information was registered in an Excel sheet and analyzed using descriptive statistics —such as mean, standard deviation, ranges, and percentages— to describe the producers and management activities.

RESULTS AND DISCUSSION

Personal Features

The average age of the 27 producers was 44.96 years (±13.7 years), the oldest producers were 68 years old, and the youngest were 21 years old. Most of the producers had approximately 35 years (Figure 1). The average age below 50 years is similar to the results reported by Oros *et al.* (2011) and Flores-González *et al.* (2019).

According to the SAGARPA-FAO (2014) criteria, the average age of the cattle ranchers in the municipality of Loma Bonita, Oaxaca, is indicative of a young rural population (up to 50 years old). This situation gains traction, because young producers are considered to be more susceptible to adopt new technologies, knowledge, techniques, etc. than old producers (more than 50 years old) (García-Salazar *et al.*, 2018; Pérez and Larios, 2018). In theory, this would facilitate the training of producers on issues related to cattle welfare, through technical assistance, in the municipality of Loma Bonita, Oaxaca.

Regarding gender, 96.3% of the participants were men and there was only one woman, which indicates that this is a male-dominated activity. Vilaboa-Arroniz *et al.* (2009) and Martínez *et al.* (2012) also report similar findings in cattle ranching from municipalities and communities of the Papaloapan region, Mexico. These results perhaps indicate that this activity involves a great deal of physical work, that management is up to a certain point carried out far from the producer's home, and that it requires investing a considerable amount of time and assuming risks, both in the herding and inside the production unit. It is also possible that the land ownership remains under a patriarchal model, in which women do not have access or control over livestock activities.

This is important because some researches (Martínez and Gómez, 2012; Barba *et al.*, 2015) had reported that the chances of adopting a technology are greater when the person in charge of an agricultural and livestock production unit is a man, because they have

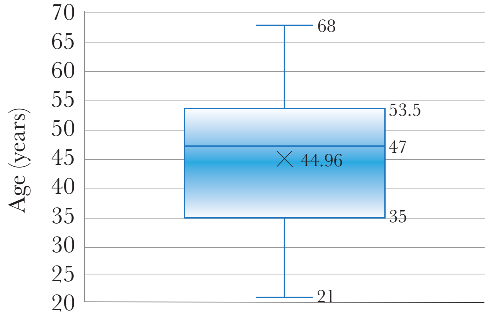

Figure 1. Age of cattle producers from Loma Bonita, Oaxaca, Mexico.

more access and decision-making power regarding the use of technologies than women. According to the results obtained in this study, this situation would also favor the spreading and adoption of practices aimed at animal welfare.

Regarding the level of schooling, 7 (25.9%) and 8 (29.6%) producers had attended elementary and secondary education (55.5% of the producers). The remaining 44.4% was distributed among those who had had attended high school (18.5%), obtained an undergraduate or postgraduate degree (14.8%), and those who did not have studies (11.1%) (Figure 2). These results indicate that more than 60% of the producers have studies above elementary school, which contrasts with the findings of Chalate-Molina *et al.* (2010), Oros *et al.* (2011), and Martínez *et al.* (2012), for dual-purpose cattle production systems under similar agroclimatic conditions, in the states of Chiapas, Morelos, and Veracruz, respectively, where the schooling average was ≤6 years. Mejía and Oliver (2005) and Flores-González *et al.* (2019) reported higher percentages of producers with elementary education.

There is a positive relationship between the level of schooling and the ability to adopt technology, since the adoption possibilities are greater at higher level of schooling (Vargas-Canales *et al.*, 2018; Cuevas-Reyes, 2019), which would also favor the promotion of technical assistance and the training of producers on animal welfare issues.

With regard to experience, 44.4% said they had been practicing cattle ranching for less than 20 years and the remaining 55.6% (15 producers) said they had more than 20 years of experience. Vilaboa-Arroniz *et al.* (2009), Oros *et al.* (2011), Torres *et al.* (2014), and other authors also report an average experience of more than 20 years. Experience in agricultural and livestock activities is also considered essential for the adoption of technologies and knowledge. Therefore, producers who have been carrying out an activity for the longest time are more likely to adopt the technology (Vargas-Canales *et al.*, 2018). This can benefit training and technical advice programs aimed at the implementation of strategies and plans that favor cattle welfare in the municipality of Loma Bonita, Oaxaca.

Animal Management and Welfare Activities

With regard to roundup methods, 74.1% of the producers said that they whistle and speak to the animals; the remaining 25.9% (7) mentioned that they hit them with sticks

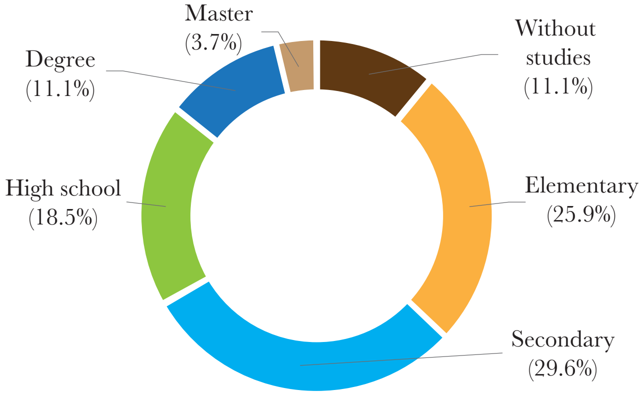

Figure 2. Level of schooling of the cattle producers of Loma Bonita, Oaxaca, Mexico.

and yell at them before taking them to be milked (Table 1). These results show the use of the three existing types of operator-animal interactions; the highest percentage belongs to the auditory and visual interactions. In general, a calm roundup could be observed in most of the ranches; the animals were docile and obedient. These observations support the findings of Herrán *et al.* (2017), who found that these interactions generate few behavioral responses and calmer cattle management.

In relation to colostrum supply, all producers declared that they helped the newborn calves to suckle; this process allows them to obtain their first nutrients outside the womb and to develop antibodies to face future diseases. The results are similar to the findings of Mejía and Oliver (2005), who reported that calves in municipalities in the Colombian high tropics obtained colostrum mainly from their mothers, followed by colostrum from a colostrum bank. Natural breastfeeding and the use of a bottle are the two methods through which they are supplied colostrum. Additionally, Reyes *et al.* (2011) suggested washing the udder of the cow before allowing the calf to suckle, to avoid the transmission of infections to the calf, as part of the colostrum supply and cattle welfare in tropical regions. Calves should be allowed to suckle as much colostrum as they wants for the first three to four days after calving. If the calf does not suck all the colostrum, the excess should be milked to prevent engorgement and damage to the udder.

Regarding the birth of the calves, 17 producers (63%) stated that they cut and tie the navel and apply methylene blue to prevent possible infections in newborns, while the remaining 10 (37%) accepted that they do not apply anything. The percentage of producers who heal the navel (67%) is lower than the 86% —regarding the healing of calves with iodine in milk-producing farms located in the high tropics of Colombia— reported by Mejía and Oliver (2005).

Twenty-one farmers (77.8%) stated that they still dehorn their animals, while the remaining 22.2% mentioned that they no longer do it. Out of the 21 producers who dehorn their cattle, 16 (76.2%) use scissors or hot iron; the remaining 23.8% apply paste. In this regard, Ordoñez and Caicedo (2016) pointed out that cutting and cauterizing with hot iron is the most painful disbudding method, which represents another area of improvement for Loma Bonita producers.

Regarding the washing or disinfection of hands before milking, 6 producers (22.2%) neither wash nor disinfect their hands; while the remaining 77.8% affirmed that they do.

Table 1. Management activities related to cattle welfare in Loma Bonita, Oaxaca, Mexico.

The producer or person in charge of the production unit…	Yes (%)	No (%)
1. Gathers the cattle hitting them when he takes them to be milked	25.9	74.1
2. Supplies colostrum within the first hours after calving	100.0	0.0
3. Ties, cuts, and disinfects the navel of newborn calves	63.0	37.0
4. Dehorns calves within the first three months of age	77.8	22.2
5. Dehorns with scissors or hot iron*	76.2	23.8
6. Washes his hands before milking	77.8	22.2
7. Washes or cleans the udder before milking	51.9	48.1

*Percentages calculated based on the 21 producers who dehorned with scissors or hot iron.

Additionally, 13 producers (48.1%) do not wash or clean the udders. These results match the findings of Calderón *et al.* (2009) and Mendoza *et al.* (2017), who warn that the lack of hygiene or and the implementation of poor practices prior to and during milking are associated with mastitis, resulting in a reduced production, increased treatment costs, and reduced milk quality. In the case of Loma Bonita, it is worth mentioning that 16 (59.3%) out of the 27 producers reported mastitis problems in their farms, which could be associated with these bad practices.

CONCLUSIONS

Based on the personal features of the 27 cattle producers from the municipality of Loma Bonita, Oaxaca, included in the sample used for this research, we conclude that there are conditions that encourage the different actors of livestock activity in Mexico (extensionists, researchers, etc.) to propose and carry out programs, plans, and strategies aimed to promote and apply good cattle practices. These programs, plans, and strategies should be focused on providing welfare within the production units, particularly in those that have human-animal interaction, including: rounding-up without hitting the animals; cutting, tying, and disinfecting the navel of newborn calves; dehorning calves; and washing and/or disinfecting hands and udders.

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AP AGRO PRODUCTIVIDAD

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Biotechnology and the potential of rhizosphere microorganisms in agricultural practices

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ABSTRACT

Objective: To perform a brief introspective regarding the biotechnological management of microorganisms in the rhizosphere, its implementation in bioprocesses, and its practical application in field.

Design/methodology/approach: Bibliographic review regarding the beneficial effects of Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR), which can be applied in bioformulations.

Results: There are numerous documented applications of AMF and PGPR —both on laboratory and industrial scale for bioformulation production— aimed to improve crop yield and to provide resistance against abiotic stress and pests. Non-conventional uses are also shown in non-agricultural areas.

Study limitations/implications: AMF and PGPR are widely recognized in agriculture due to their inherent ability to compete in harsh conditions within ecosystems, metabolism versatility, and production of secondary metabolites that enable beneficial interactions with plants and other microorganisms. However, industrial production of AMF presents challenges, as a result of their obligate biotrophs condition and a lack of compatibility with traditional bioprocesses.

Findings/conclusions: The knowledge generated throughout rhizosphere research should be applied in the industry, in order to extend its use in agriculture.

Keywords: Biotechnology, Bioprocess, Industrial microbiology, Arbuscular mycorrhiza, Plant growth-promoting rhizobacteria.

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INTRODUCTION

Currently, awareness regarding the risks of agrochemicals has experienced a considerable increase. These materials are regulated and even banned in some countries, because their use has adverse side effects on the environment, the biota, and human health. As a result of the changes that their uncontrolled use has caused to the ecosystems, a search for a method that improves crops conditions, without recurring to chemical products, have been undertaken. From the point of view of the soil ecosystems studies, the knowledge about the

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interaction of plants and rhizosphere microorganisms offers a viable alternative to improve agricultural practices, not just increasing fertility, but also providing plants with resistance against pests and abiotic stress.

In the context of agricultural practices, the biotechnical management of rhizosphere microorganisms in the field is compatible with organic agriculture techniques. The market offers bioformulations —which use plant-growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF)— to improve crops; therefore, the need to implement more technologies related to favorable biotic interactions is increasing and opens a niche to implement new developments or to improve existing developments.

Biotechnology aimed at the application of microorganisms in agriculture

Biotechnology is an interdisciplinary approach that uses live organisms to obtain products of overall interest for human activities. This technology uses algae, bacteria, fungi, yeast, plant and higher animal cells, or subsystems of any of them, as well as isolated compounds of live matter (Bhatia and Goli, 2018). This discipline is as old as civilization itself: it allowed the development of plants, the domestication of animals, and the production of food —including cheese, yoghurt, bread, vinegar, beer, and wine (Arfin and Sonawane, 2019). Biotechnology includes several areas that can or cannot involve genetic characterization or manipulation. Although progress in plant and microorganism genetic engineering has benefited agriculture, there are areas that do not involve genetic manipulation —such as classical biotechnology and bioprocesses— and which have great potential, mainly for the improvement of the quality of agricultural products and crop productivity.

Biochemical or bioprocess engineering enables the development of processes and equipment that provide products of interest —mainly on an industrial scale— through fermentation processes that seek to produce greater quantities and achieve a higher quality of a particular product (cells, spores, primary or secondary metabolites) (Doran, 2013). Industrial microbiology is the area of knowledge that studies microorganisms that are handled through bioprocesses. This area deals with large-scale fermentations and compensates the limitations for their application, such as substrata cost and quality, operational costs, purity of the starter culture, nutritional needs of the organisms, product purification, product yield regarding the substrata, and non-optimized metabolic pathways (Behera *et al.* 2019).

Within the procedures to establish a bioprocess, a strain must be chosen —whether from the environment or a collection—, in order to obtain a highly-concentrated product of interest. Subsequently, the process must be optimized: first in the lab and then at a pilot scale, before escalating it to an industrial level. Optimization means a fair and appropriate use of resources, in order to obtain the maximum potential yield of the desired product. Fermentations are influenced by such variables as: agitation, ventilation, temperature, and pH, as well as more specific elements, depending on the microorganism used for this purpose. All these systems have operational costs —such as power, fuel, water, and maintenance, among other supplies and expenditures— that directly influence the profitability of the process.

Industrial microbiology has been interested in rhizosphere microorganisms, because their application is relevant to sustainable agriculture (Barea, 2015) and non-agricultural areas. As a result of their favorable interaction with the ecosystem, they promote plant growth and long-term pathogen control, reducing the use of synthetic fertilizers and pest-control substances.

Interactions between the plant roots and the rhizosphere microorganisms take place when the plant attracts the symbiotic organism, which interacts with its root. In its turn, the microorganism must distinguish itself as a symbiotic organism, rather than a pathogen; subsequently, it gains a regulated entry into the root. The carbon photosynthetically fixed by the plant (photosynthates) is directly transferred to the below-ground biomass through root exudates, providing nutrients for epiphytic and saprophytic organisms —which accelerate the decomposition of the soil organic matter— or endophytic organisms —which are associated to the plant cells, through N-fixing microorganisms or mycorrhizal symbiosis (Horwath, 2007).

This results in a situation where each organism must take part in a complex and structured communication that enables the successful establishment of the symbiosis. The term "molecular dialogue" refers to chemotaxis and was coined to describe the communication between the plant roots and the microorganisms (Perotto and Baluška, 2012). This process takes places through the production of secondary metabolites, which have different functions, including: signaling molecules, growth regulators, organic acids, enzymes, antibiotics, biopolymers, etc.

Biotechnological management of arbuscular mycorrhizal fungi and significant challenges

The arbuscular mycorrhiza (AM) is made up of fungi which penetrate the vascular plant roots cortical cells; AM is an advantage for vegetal growth. They promote the absorption of mineral nutrients and water, because their hyphae —which are thinner than the roots— can penetrate small pores that are inaccessible to the roots (Allen, 2011). Not only does AM improve the nutrients that the plants receive, it also enhances their resistance to abiotic stress (salinity, drought, and floods), as well as their resistance to diseases (Berruti *et al.*, 2016). The AM contributes to the degradation of organic matter producing extracellular enzymes and to soil stabilization producing proteins, such as glomalin and hydrophobin (Leinweber *et al.* 2013). AMFs are considered primary biotic compounds of the soil; therefore, the absence of mycorrhizas is considered abnormal for most vegetable species, resulting in an inefficient functioning of the ecosystem (Jansa and Gryndler, 2010).

AMFs are obligate biotrophs, because they must establish an association inside the plant roots to produce propagules; therefore, the common bioprocesses for the heterotrophic microorganism culture are not favorable for this type of fungi. The AMF production is carried by the plant and its symbiont in different systems: **1. Substrata culture**: sand or perlite —in bags, pots, or containers inside a greenhouse—, in non-sterile conditions; it does not require complex techniques and has a low cost, although it is difficult to keep it safe from pollutants and particles (Millner and Kitt, 1992). **2. Substrata free**: hydroponic and aeroponic ecosystems, which use nutritive solutions and require precolonized plants.

These systems allow an improved spore harvest and reduce the risk of contamination; however, they have disadvantages, such as the greater complexity of the infrastructure and the high risk of bacteria and algae contamination of their solutions (Ijdo *et al.*, 2011). 3. ***In vitro* AMF culture in tissue (monoxenic culture)**: this system may or may not require transformed roots to produce propagules. It prevents contamination throughout the process and enables a more efficient implementation, in a greater scale than the previous two systems (Kokkoris and Hart, 2019). The disadvantages are its high costs and the need for highly-qualified technical staff.

Hard work is being carried out to improve the AMF culture conditions. Currently, no processes that allow the large-scale, low-cost propagule production are available. This is a huge disadvantage for its industrial production and its large-scale application in the field.

Although there are different types of reactors for cultures submerged with mechanical or pneumatic agitation (airlift reactors), they have adverse side effects, such as root asphyxia. This is the result of the low solubility of oxygen in liquid environments (1.22×10^{-3} mol dm^{-3} at 25 °C at 1 atm in pure water) (Xing *et al.*, 2014), which causes root asphyxia and the loss of feasibility. In a liquid culture environment, the lack of oxygen is usually compensated with enough aeration (which also works as an agitation in the airlift reactors) and an increase in the power of the agitation (for reactors with a mechanic agitator). In vegetable tissue cultures, the conditions for the bacteria and filamentous fungi cannot be used, because they produce cell stress and the AMF are not adapted to the said culture conditions. The approach for AMF cultures consists in other techniques which send oxygen to the roots, whether they have been transformed or not, in similar conditions to those of their natural environment, but retaining contact with the culture medium, without producing root asphyxia. Table 1 shows several patents regarding AMF bioformulation production for its application in the field. These bioformulations are made up of spores harvested in *in vitro* production conditions. Additionally, a proposal to produce AMF propagules in roots —whether they have been transformed or not— using a gas-phase reactor was made. The international WO2019/003240Al patent suggests that, in order to achieve a proper root oxygenation and nutrition, they should be in contact with the disperse culture medium as fog, in a closed system under sterile conditions.

The importance of bioinput for agriculture

Bioinputs are currently being used as an alternative or complement to conventional agrochemicals. According to their functionality, they are classified as: bioinsecticides, biotech insect repellents, biofungicides, biofertilizers, biostimulants, pest-control substances, and inoculants. This type of preparations may include substances that achieve a desirable nutritional effect or an efficient pest and disease control; they can include plant, mineral, animal, and microbial elements, and even living or latent microorganisms. Inoculants may contain a single or a combination of microorganisms. Their formulas can include other substances. Depending on their effect, they can also be classified as biostimulants, pest-control substances, and biofertilizers.

Table 1. Examples of patents that implicate the use of AMF in bioformulations and technology for the production of propagules in bioreactors.

Patent / Protection	Development	Country/Year/Applicant	Description	Species	Amount of propagules
WO2019/003240Al / Internacional	A novel bioreactor for mass production of arbuscular mycorrhizal fungi	India /2019/ The Energy and Resources Institute	Gas state bioreactor: fungal spore culture and transformed roots in an aseptic space, with the culture medium dispersing as a mist, recirculating the condensates	Not specified	It does not specify operating capacity; it only ensures to obtain roots in 4 weeks
US 10,362,787 / Estados Unidos	Method for propagating microorganisms within plant bioreactors and stably storing microorganisms within agricultural seeds	United States /2019/ Indigo AG, Austrian Institute of Technology GMBH	Cultivation of endophytic organisms in plant tissues and their storage in seeds to improve their shelf life	Glomeromycota, mezclas de organismos de biocontrol y rizobios	Not specified
US 10,238,103 / Estados Unidos	Rhizobia and mycorrhizal granular formulations and mixtures thereof	United States /2019/ Valent Biosciences LLC	Formulations with mycorrhizal propagules	Not specified	Rhizobia: $1x10^{-9}$ UFC/g AMF propagules: from 200,000 to 600,000 per gram
US 8,883,679 / Estados Unidos	Liquid mycorrhiza compositions	United States /2014/ Novozymes A/S	Formulations with mycorrhizal fungi propagules	*Glomus aggregatum*, *Glomus brasilianum*, *Glomus clarum*, *Glomus deserticola*, *Glomus etunicatum*, *Rhizoglomus fasciculatum*, *Rhizophagus irregularis*, *Glomus monosporum*, *Glomus mosseae*, *Gigaspora margarita*	(Myco Apply Ultra®) 286 propagules per gram, dry granules or moisturizable powder

The inoculant production process includes several stages: isolation of the strains with the best characteristics for the objective; efficient lab and field tests; fermentation methods; formulation viability; choosing the appropriate carrier; toxicology; industrial scaling; and quality control (Xavier *et al.*, 2004; O'Callaghan, 2016). Quality control is an aspect that must be particularly emphasized. Several aspects that are included in the regulations of several countries around the world must be taken into account, including: crop effectiveness and persistence; viable microorganism count in the product; survival of the organisms in the carrier; shelf life; carrier sterility; and compatibility with native microbiota. In Mexico, these aspects are governed by the NOM-077-FITO-2000 (https://www.gob.mx/senasica/documentos/nom-077-fito-2000) standard, which is currently being modified.

Biotechnological management of plant growth-promoting bacteria (PGPB)

More and more mechanisms through which bacteria favor the promotion of plant growth are discovered every day. They can facilitate the availability of certain nutrients in insoluble forms —for example, in the release of siderophores and organic acids or the fixation of atmospheric nitrogen—, they diminish the competition for the substrate, they produce growth-regulating substances, and they interfere with the signals of antagonistic organisms, etc. (Souza *et al.*, 2015; Ramakrishna *et al.*, 2019). This type of mutualistic interaction allows the plant to improve its conditions, while the microorganisms increase their chances of surviving in a highly competitive environment. PGPB benefit their hosts through various action mechanisms, depending on the species and the colonization and development conditions.

Since survival in the rhizosphere faces highly competitive conditions, bacteria have an extremely high capacity to produce extracellular secondary metabolites (enzymes and other molecules) and to make the most of substrates from several sources and complexities (Sant'Anna *et al.*, 2011; Eida *et al.*, 2020). Table 2 shows various ways in which bacteria groups can be used for agriculture and other non-conventional activities (such as the production of industry-focused metabolites). This versatility is highly appreciated in practical applications, both in agriculture and other areas, including: bioremediation, biorefining, production of food, make-up or medicine additives, production of biopolymers, enzymes, and biosurfactants, etc.

The production of PGPB for their use as inoculants is an alternative to agrochemicals. Additionally, it leads to products with higher quality and greater yield, both individually and mixed with other organisms, such as AMF (Mishra and Arora, 2016).

Gómez-Merino *et al.* (2014), Ferrera-Cerrato *et al.* (2016), Mitter *et al.* (2021), and other researchers have highlighted the importance and need of soil microbiology knowledge to provide greater support for plant nutrition and agricultural production, from a sustainable and innovative point of view.

CONCLUSIONS

The rhizosphere microorganisms offer promising solutions to achieve a productive, sustainable, and eco-friendly agriculture; they also contribute to the bioremediation of polluted environments and the production of metabolites of interest for non-agricultural

Table 2. Examples of the application of plant growth-promoting bacteria, for both agricultural and non-conventional uses.

Area	Use	Genus or species	Origin	References
Bioremediation (Degrading bacteria, plant growth promoters and phytoremediation by other means)	Degradation of non-halogenated aromatic xenobiotics. Degradation of polycyclic alkanes in conjunction with plants. Phytoremediation facilitators.	*Bacillus thuringiensis* *Pseudomonas* sp. *Azotobacter* sp.	• Soil from chemical factories • Soil contaminated with hydrocarbons and heavy metals • Soil samples	Marchlewicz *et al.* 2016 Glick, 2010 Ma *et al.*, 2011
Production of exopolysaccharides (Homo and heteropolysaccharides)	Additives in food, pharmaceutical, cosmetic, oil, agricultural formulations, among others. Encapsulating agents, film formers and rheology modifiers.	*Agrobacterium*, *Rhizobium*, *Alcaligenes*, *Azotobacter vinelandii*, *Sphingomonas paucimobilis*, *Pseudomonas elodea*, *Xanthomonas campestris*.	Epiphytes or endophytes of different plant species	Figueiredo *et al.* 2016
Production of biosurfactants (Lipopeptides and lipoproteins, phospholipids, biopolymers and surfactant antibiotics)	Less toxic, more specific and biodegradable surfactants. Synthetic surfactant substitutes.	*Streptomyces roseosporus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Acinetobacter calcoaceticus*.	Soil samples	Panjiar *et al.* 2017
Biocontrol agents: production of antibiotics, siderophores, high colonization and induced systemic resistance	Advantages for plants against pathogens.	*Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Bacillus thuringiensis*	Soil samples	Salomon *et al.* 2017
Biofertilizers (rhizospheric and endophytic)	Production of growth regulators, nitrogen fixation, production of siderophores, solubilization of phosphorus, production of organic acids, production of antibiotics, induced systemic resistance.	*Allorhizobium* sp., *Azorhizobium* sp., *Bradyrhizobium* sp., *Mesorhizobium* sp., *Sinorhizobium* sp., *Azospirillum* sp., *Pseudomonas* sp., *Bacillus* sp., *Azotobacter* sp.	Soil samples. Endophytes of different plant species.	Bhattacharyya *et al.* 2012

areas. Biotechnology and its various branches provide alternative solutions to problems such as: crop quality, pollution, and the extensive use of agrochemicals. Specifically, they contribute to the establishment of bioprocesses related to the massive production of microorganisms whose effectivity for their extensive use in the field has been proven, depending on the problem that needs to be solved. Therefore, the knowledge about rhizosphere microbiology must establish a close collaboration with agricultural producers and the industries, in order to develop feasible large-scale solutions.

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AP AGRO PRODUCTIVIDAD

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Strategies and socio-productive relationships in pig backyard production

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ABSTRACT

Objective: To determine socioeconomic relationships in backyard pig farms.

Design/methodology/approximation: The farms studied had 1 to 8 sows. We used semi-structured questionnaires and periodic monitoring to collect data. We determined the cost of production with the general cost formula and calculated the countable effect of family labor. Two groups were established: Group 1) Young married couples; and Group 2) Elderly couples who have been married for a long time and who do not have married children. Social Network Analysis was used to determine the strength of those relationships.

Results: Five socio-productive relations were discovered: 1) input supplier-producer; 2) piglet breeder-swineherd-butcher; 3) religious festivities-pigs; 4) season of the year-pig; and 5) producer-boar keeper. Income differed when family work was not counted.

Limitations/implications: Results showed that it was possible to combine qualitative data and statistical modelling in studies about social and economic behavior of backyard pig farms and small-scale farms.

Key words: Economic well-being, economic sustainability, livestock, social network analysis.

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INTRODUCTION

Neoliberal development models —which always seek greater competitiveness— generated enormous pressure on regional agri-food systems (Ploeg, 2010; Delgado, 2010). However, the socio-structural complexity of these systems (their constitution and dynamics), involve elements that do respond to both commercial and community criteria (Rendón *et al.*, 2019). The structural basis of agri-food systems is purely social, since its function is to feed societies by producing and transforming natural inputs (Steinfeld *et al.*, 2009).

Backyard pig farming is a highly differentiated sector in the various regions and communities throughout the world. Each livestock agri-food system is developed in a

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particular way, in terms of the species, stocks, and inputs that are used, and the production method (Steinfeld *et al.*, 2009; FAOSTAT, 2017). According to FAOSTAT (2020), the 2019 world pig inventory was just over 850 million heads (Mexico contributed 2.16%), constituting the main source of animal protein worldwide. In Mexico, estimates suggest that backyard production represents 10 to 30% of the inventory and contributes just over 30% of the total meat produced. However, this system has been described as not particularly functional, as a consequence of its low productivity, deficiencies in the design and planning of the establishments, and the lack of training of the producers, among other characteristics. Despite these deficiencies, these low-technological, -energy, -economic, and -environmental systems (Martínez and Perea, 2012) are reservoirs of genetic variation (Martínez *et al.*, 2016; Montero and Martínez, 2016), support the food (and culinary), job, edaphological, and socioeconomic security of the communities (Brunori *et al.*, 2012; San Vicente, 2018; Santos-Barrios *et al.*, 2019) and produce high-quality protein (Velázquez-Villalva *et al.*, 2016).

Backyard pig farming includes production systems in periurban areas which have assimilated some characteristics of urban consumption, but which have kept economic, social and cultural dynamics that belong to the rural environment (Fernández and Morán, 2015; Hernández-Puig, 2016). Fernández and de la Vega (2017) point out that 43% of the population is involved in agricultural production —more than 70% of which is destined for self-consumption. Likewise, they are part of the family financial strategies of the users (Santos-Barrios *et al.*, 2019) and their socioeconomic resilience is a result of the convergence of such aspects as: "productive diversity, recycling of resources, cooperation, and mutual support" (López *et al.*, 2012).

The family livestock production units can be considered as integration nodes with various strategies to face scarcity and achieve a certain livelihood (Rivera *et al.*, 2015). Therefore, they are framed in complex socioeconomic compositions that allow farm family units to obtain a certain security, potentially impacting the development of an entire region (Montero and Martínez, 2016). The organization of the backyard implies the presence of multiple, diverse, and interrelated actors and (social, technological, and economic) factors; its particular management is determined by the psychology (interests, needs and resources) of the farmers themselves (Martínez and Perea, 2012).

The backyard means developing a structural discourse where products, strategies, inputs, and actors have an impact on the said social structures to which producers turn to in order to feed the herd. In the highly-competitive agro-industrial field, these types of extremely fragile structures are continually subject to a disruption process. For example, they face high and irregular production costs (Hernández-Martínez *et al.*, 2008), which are a sign of the inconsistent social structures to which the backyard pig farmer must turn to, in order to obtain the necessary inputs. Hence, the need to expose its complexity and socio-structural fragility.

In this context, the objective of this work was to determine the socioeconomic strategies and the relationship structure involved in backyard pig farming, in a determined rural community in Mexico, and to establish the resulting income of the producers.

MATERIALS AND METHODS

This study was carried out in the community of San Miguel Coatlinchán, of Texcoco, State of Mexico. This town is located 9.4 km away from the municipal capital and 24 km away from Mexico City. It has an altitude of 2,250 masl, a temperate semi-arid climate, an average annual temperature of 15.9 °C, and an average annual rainfall of 686 mm (INAFED, 2016).

Data collection

An ethnographic approach was used to tackle the social structure and the problem itself. The sampling frame was developed based on the lists of producers who participated in previous research (Santos-Barrios *et al.*, 2017), which were refined through periodic visits to livestock farms.

Forty-five backyard pig farms were studied from August 2015 to August 2017. We included farms that had from 1 to 8 breeding sows. Productive, social, and economic indicators were obtained.

We proposed an analysis by age groups to obtain a clearer visualization of the dynamics and strategies.

Analysis of data

The production costs of a weaned piglet were determined (Muñoz and Rouco, 1995). The analysis included the opportunity cost of family labor (Bobadilla-Soto *et al.*, 2013).

The data were analyzed using descriptive statistical techniques and frequency analysis. An analysis of variance was used to compare variables between groups and the differences were analyzed with the Tukey Test.

According to the default network unit of analysis (producer-input) and the available information, organizational structures referred to by the producers of the analyzed population were extracted and the degree centrality of the said relationships was measured.

Data was collected through semi-structured interviews, complemented by periodic monitoring and recording of the above-mentioned indicators.

RESULTS AND DISCUSSION

The pig farmers of San Miguel Coatlinchán have adopted production and distribution strategies that provide them with economic benefits, such as the sale of piglets with 40-day lactation periods, which allow them to sell heavier and more viable piglets, meeting the requirements of the buyers. Unsold piglets are fattened until they reach commercial weight and size (90-100 kg). These specimens can be sold in three ways: live, carcass, and processed meat (fried with lard).

The sale price of a weaned piglet was $33.68 USD. The monetary values are expressed in American Dollars (USD) with an exchange rate of 1 USD:17.8145 Mexican pesos, as of August 31, 2017. When the cost of family labor (SMOF) was not taken into consideration, average costs were $20.99±3.03 USD, with a maximum cost of $25.76 and a minimum cost of $16.50. The average cost of a weaned piglet, after taking into consideration the cost of labor (CMOF), was $24.02±3.48 USD, with a maximum cost of $33.62 and a minimum cost of \$18.13. The average CMOF profit was \$12.68±3.03 USD, with a maximum profit of \$17 and a minimum profit of \$5.89 and of \$9.54±0.02 USD, with a maximum profit of \$15.54 and a minimum profit of \$0.06, when CMOF was considered.

Backyard pig production in San Miguel Coatlinchán is organized on pre-established social structures, such as the family and the community. This is a family economic activity, in which all the members take part, depending on their age, physical, capacity, and gender. Based on the informants' narrative, we can infer that this backyard pig production model has been developed and reproduced for at least 150 years, which implies that between six and eight generations have made a cultural investment in its social reproduction (Martínez-Castañeda and Perea-Peña, 2012). The fact that all members of the family, be it nuclear or extended, are involved in the tasks that the activity imposes, ensures its social reproduction (Ruiz-Torres *et al.*, 2017). The producers reported that they sell both to intermediate consumers and to final consumers. Other members of the community are always the final consumers. However, producers prefer to sell to intermediate consumers (middle men), who buy all commercial-size pigs from their farms. It is worth mentioning that most of these intermediaries have their own butcher shops outside the State of Mexico, in other states such as Michoacán, Puebla, and Tlaxcala. Commercial transactions between producers and middlemen in these states place the former in the lowest link of the Agrifood Value Chain (CVA). According to Gonzales-Razo *et al.*, (2014), the middlemen determine the final price of meat by 74%.

Pig farmers by age groups

In order to have a more refined perspective on the social dynamics that are generated within this type of production, two age groups were established: 1) young couples with children; and 2) elderly couples with unmarried children.

1. This group was made up of people older than 25 years; 76% of the producers in the sample belong to this group. In the case of young couples, women usually tend the farm, while men work as formal employees, thus ensuring a regular income and access to social security. The incorporation of children around the age of 10 to farm work —carrying out minor, but still important tasks— in this first age group is a relevant characteristic that would help us to understand the generational change and the social reproduction of this economic activity. Child labor (caring for animals) becomes more complex as minors grow and acquire more experience. When young people are 17 years old or older, they spend more than 80% of their free time taking care of the farm; however, this does not give them the right to make management decisions. The parents are always and at all times responsible for the decisions regarding the investment in the farm and the marketing of the animals. Usually, ten to fifteen years after the children begin to work in the family farm, they achieve their independence from the house —and therefore from the farm. During this time, young people learn about the activity, develop the skills and abilities necessary to operate and manage a farm, and incorporate this activity into their livelihood strategies; this third element

guarantees the social reproduction of this economic activity. When a child becomes and adult and gets married, a change in family and social status takes place. When the son becomes head of the family, the parents help him to start his own farm. If the newly-weds do not have enough physical space to have a farm, the parents lend their son a pen. The son is expected to start raising pigs independently, initiating the social reproduction cycle of this activity.

2. The most outstanding phenomenon of the second age group, is the "return" of the husbands to the home: many of them retire, while others formally stop working and begin to "work" on the farm. Twenty-four percent of the producers in the sample belong to this group. In this group, the heads of families take up again the activities and management of the farm. Unmarried children of productive age allocate resources for the family economy, providing external financing for the farm.

Likewise, farms run by elderly couples were identified, where the fundamental workforce consists of grandchildren, nephews, and young relatives. These elderly producers are no longer the family's economic support, but they continue to fulfill an important social role as a source of experience and family cohesion. Very old producers usually become part of their son's family, to whom they transfer the work of their farm. The son is responsible for the maintenance and care of his parents, but at the same time, the farm of the elderly producer remains in operation, therefore allowing him to provide income to the new family nucleus. Consequently, he does not perceive himself as a burden and maintains his dignity, as an individual useful to the family and social group.

Although backyard pig production is not the main economic activity of the family nucleus, it does constitute an important income supplement that enables the family to overcome eventualities. The main headings to which the resources obtained from production are allocated are: a) annual school expenses, such as enrollment, school supplies, school uniforms, etc.; b) medical expenses; c) clothing and footwear; and d) civil, religious, and family festivities, in which the pigs themselves are used to prepare the food. No significative difference —resulting from age or schooling—was found ($P>0.05$) between the two groups, with regard to productivity, income, or herd size.

In this study, regular self-consumption was only observed on large family festivities.

Relations of backyard pig production with other economic activities

Although family farming activities fail to establish a link with the national Agrifood Value Chain (CVA), they generate local and regional links, before and after the said production. Backyard pig production, like any other family farming activity, generates links with other economic activities and various social actors. Key relationships were identified, which are fundamental for the successful operation of the activity:

1. Relationship between input suppliers and producers: the relationship established by the producer with the suppliers of the diet ingredients in the different stages of production. The first stage happens at the same time as the gestation period of the sow. The commercial exchange is established, on the one hand, with forage farms —

where the producer-forage farm relationship is direct— and, on the other hand, with bakeries and cake shops —where the producer-bakery relationship is supported by a middleman who obtains the by-products and sells them to the producers. However, it is not an open business relationship as the middleman sells to a specific group of producers. Likewise, the relationship extends to households, where the producer-household relationship is a closed relationship, since household members only give food waste to relatives or friends. The second stage takes place during the piglets' suckling stage. On the one hand, the nutritional requirements of the sow and the amount of feed consumed increase and, on the other hand, from 30 days of age the piglets are only provided fodder whose forage-production relationship can be identified. Given that the average lactation lasts 40 days, the social implications and relations between producer and suppliers are of vital importance; other actors do not place their product; this relationship is activated when the piglets are sold. The distributor and the producer have a sound trust relationship, as this ensures that good quality ingredients are always available at an affordable price.

2. Relationship between piglet breeders, swineherds, and butchers: The piglet breeder-swineherd relationship is a power relationship where the swineherd has the advantage: the lack of a deal or agreement for the purchase and sale of piglets means that the swineherd can buy half or a full litter at will. Consequently, the piglet breeder develops strategies, such as keeping one or two underweight piglets to fatten them and sell them later. We should point out that local consumers appreciate this type of production and they look for butchers that sell "clean", local, and tasty meat.
3. Relationship between religious festivals and pigs: Thirteen religious festivals were identified in the community of San Miguel Coatlinchán —of which Saint Michael (their patron saint), Holy Week, and Christmas are the most important—, in all of which a meal is offered to the faithful. This meal can include *carnitas*, chicken, turkey, etc., depending on the "tastes" of the patron who pays for the festivity. During the festivities of Saint Michael and Christmas pork is the traditional dish. The patrons of these festivities ask a swineherd to put pigs on lawaway or fatten them themselves. However, the swineherds depend on the suckling pigs providers to fulfill the task. The relationship between piglet breeders, swineherds, and religious festivals is supported by long-standing relationships between the patrons of the festival and the swineherds.
4. Relationship between the time of year and the pigs: As demand rises in December or the end of the year, butchers seek to buy and sell meat from community farms every third day. The rest of the year they do it every fortnight, selling it as fried meat and pork scratchings. This relationship makes some producers schedule their sales based on the needs of the swineherds, who are ultimately the ones who sell the pigs to the butchers.
5. Relationship between producers and boar keepers: This relationship is established between the piglet producer and another producer who has a boar in his herd, which the latter "rents or lends" to the former's farm for an average of 3 days, during which it provides reproductive service to the females that are on heat. Renting a boar costs

> $11.23 USD; the food expenses are borne by the person who receives the borrowed animal. Sometimes the mating is not paid in cash: instead, the owner of the boar agrees to choose, at the time when the piglets are weaned, females that will serve as replacements in the breeding boar farm or piglets that will be fattened for sale; optionally, the payment could be exchanged for a favor later on. Producers who own boars have access to different farms. Therefore, they have information about farms, including: production parameters, technology used, different ingredients used in diets, presence of diseases, and deficiencies. They are often an important medium for the transmission and adoption of information and technology.

Small-scale pig production in the study area is of utmost importance for the family economy, since it provides additional economic income that allows them to access material assets and services that would otherwise be impossible to obtain. Godínez-Montoya *et al.* (2015) point out that most agricultural households seek to diversify their subsistence base as a risk-reduction strategy. Likewise, they also point out that most non-farm income or self-employment are increasing. The rural population increasingly carries out non-agricultural activities, such as local commerce, the production of handicrafts, the extraction of raw materials, ecotourism, environmental services, or wage-earning work in various occupations, among others. The ability of households to develop livelihood strategies largely depends on their assets and how these are used to achieve a balance and a certain level of satisfaction (Gómez-Demetrio *et al.*, 2013).

Backyard pig production: a network structure

Backyard pig production exists and resists thanks to these community ties; otherwise, it would not exist, not even as an isolated unit. In fact, a backyard production unit implies social conglomerates through which it receives various types of inputs. A "unit of production" only exists as an autonomous entity in the statistical universe, not in the rural sociological notion of social construction, where social networks are established to provide coherence to this type of production (Figure 1).

Social structure of nutrients

Martínez-Castañeda and Perea-Peña (2012) described a network structure generated by the materials and ingredients mentioned by the analyzed producers (Figure 2A), where concentrated feed stood categorically over corn, bakery residues, tortilla, and swill in general, pointing out that "...the use of ingredients is more a situation of customs and logistics" (Martínez-Castañeda and Perea-Peña, 2012). When the degree centrality measure was applied to this network, assuming that backyard pig production mainly obeys a management structure for this type of nutrients, we observed that concentrated feed is, indeed, the actor that links and influences the pig production language; however, the second variable that links this system is swill, and thirdly corn, among others. This allows us to suppose the existence of a social structure that —in terms of the management of this type of inputs— builds a particular network of food stores and places where swill is collected (regularly, inns, restaurants, markets, etc.).

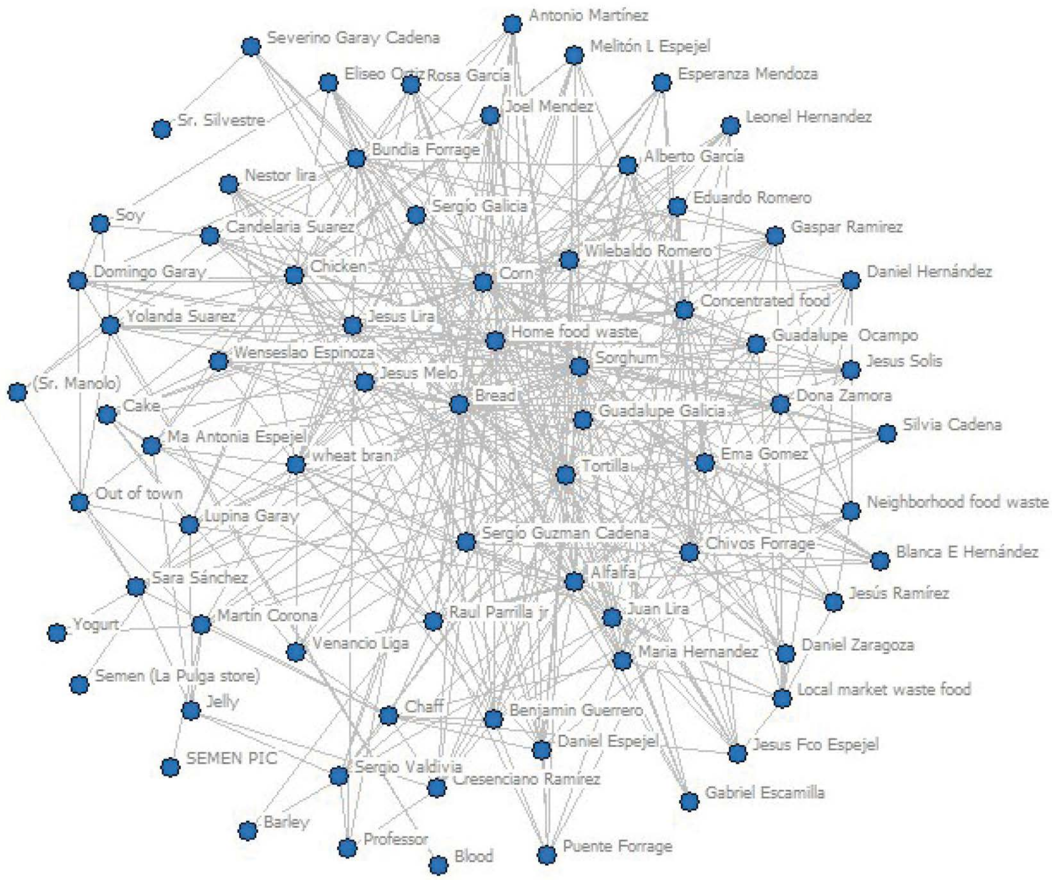

Figure 1. General structure of social inputs for backyard pig farming in San Miguel Coatlinchán, Texcoco, Mexico.

Therefore, regarding the nutritional input management structure of this study, we observed that 61.47% of the references of nutritional sources were: swill (16.71%), bakery residues (12.75%), sorghum (11.05%), tortilla waste (11.05%), and corn (9.92%). The centrality of the actors refers to a nutritional language different from the previous network, which is mounted on a network of places where swill is collected (inns, restaurants, markets, etc.), along with bakery waste, sorghum, waste tortilla, and corn. According to the resources available for the nurturing of the herd, these nutritional languages are constantly changing; hence, even in the analyzed communities, this will change for the next herds (Figure 2B).

Supply social structure

According to the physical structure of some of the supplies indicated by the producers, an image was obtained that refers to a support network —social, rather than commercial (Figure 3). Although commercial establishments have a certain importance in the supply of inputs, the main degree of centrality was presented by swill, which is usually almost all given away, although it could be commercialized. This implies a two-way community social bond: on one hand, management of urban solid organic waste; and, on the other hand, the transformation of the said solid waste into high-nutritional products with a considerable economic value (Martínez and Perea, 2012).

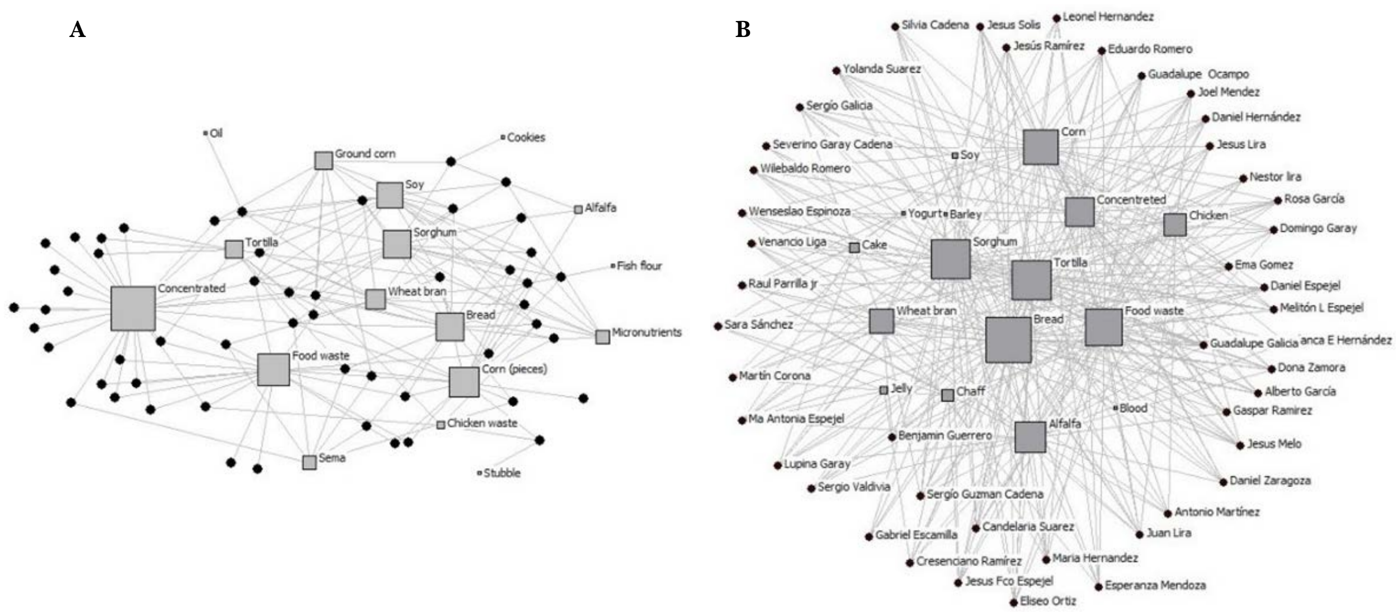

Figure 2. A) Nutritional ingredient degree used by producers (developed with data from Martínez-Castañeda and Perea-Peña, 2012); B) Nutritional ingredient degree used by producers in the study.

Social structure of boar keepers

One of the most complex social structures is generated by the exchanges of the local pig seed stock, since these structures play a major role in the maintenance of the reservoirs of genetic variation, even for commercial productions (Martínez *et al.*, 2016; Montero and Martínez, 2016).

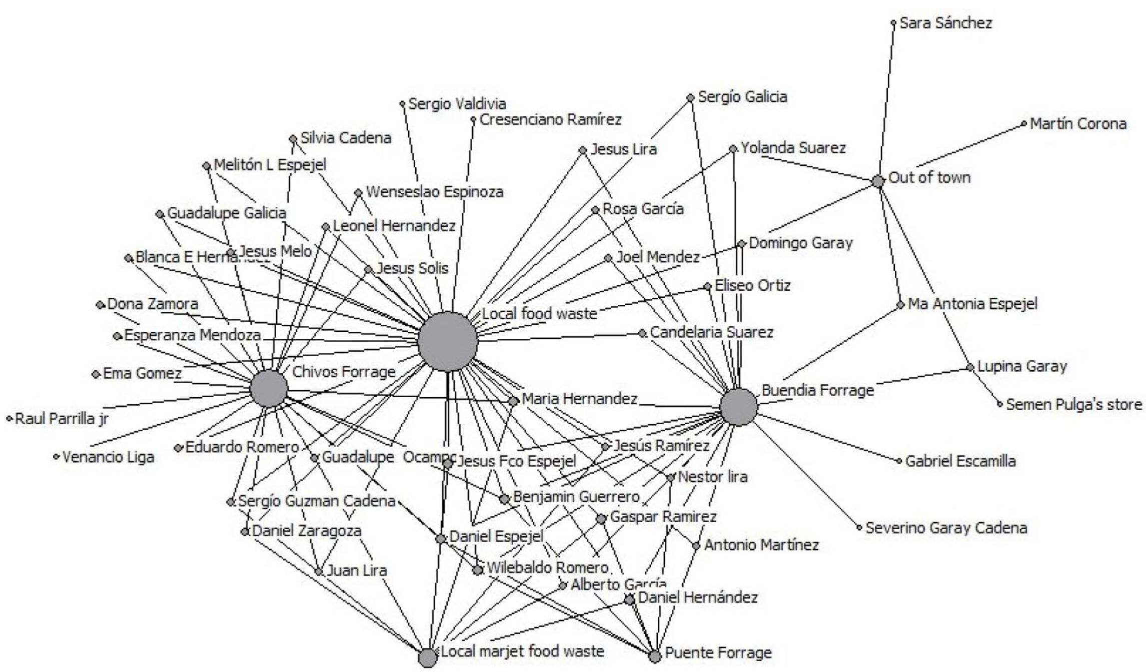

Figure 3. Network structure degree of input supply sites for pig production in San Miguel Coatlinchán, Texcoco, Mexico.

It should be noted that, at some point, the population of pig farmers analyzed acquired a great amount of seed stock from PIC (Pig Improvement Company), the international swine genetics company. The idea behind the purchase was to increase meat production; however, the results were not as expected, since this technological package must be accompanied by other specific inputs that, given their socioeconomic characteristics, are not accessible to backyard pig farmers.

In this context, the analyzed network structure (seed stock exchange) had three reticular behaviors (Figure 4).

1. Sociocentric component (green box): It is the most complex social values structure, where most of the producers can be found. The following values were observed: trust (a producer works with a single boar keeper), negotiation (a producer obtains seed stock from two or more boar keepers), and concentration (a boar keeper concentrates several producers).
   The presence of potential local seed stock circuits is highlighted, which could potentially represent a much larger and more complex circuit in the dispersion of the biological assets.
2. Triads in an intransitive state with centralized prominence (yellow box): In this type of structures, a remarkable situation takes place: a single boar keeper works with several producers. Regularly, they are recognized boar keepers in the community.
3. Intransitive state dyads with centralized prominence: These are the simplest types of structures and usually indicate relevant relationships.

Considering the service that boar keepers provide as part of a social and commercial structure —in which a particular input is managed—, the prominence relationships show the major actors of the said structure. Therefore, they are determining nodes in the

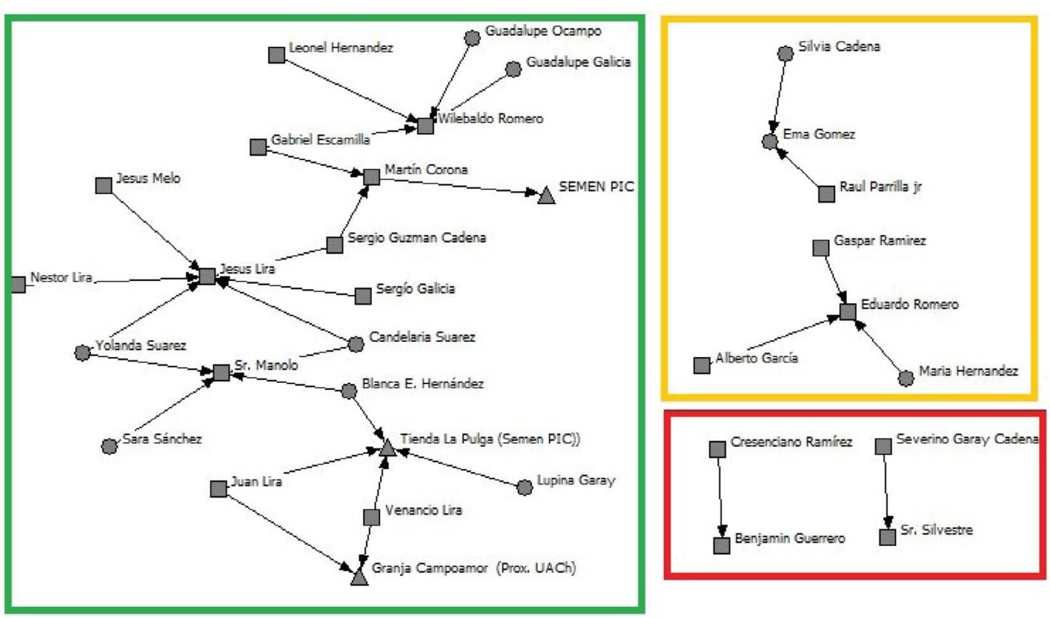

Figure 4. Social structure in boar keeper services in San Miguel Coatlinchán, Texcoco, Mexico.

said structure. In this case, they can be key actors for the analysis of this type of social complexities (Figure 5).

CONCLUSIONS

In rational terms, backyard pig production is not a matter of capitalization and/or savings by the producer, but rather a defined economic activity: the sale of piglets and the slaughter of adults are scheduled. They depend on defined expenses (emerging activities that require immediate payment): tuition fees, purchase of medicines, scheduled financial activities, self-financing, etc. And the qualities of this type of livestock production are sustained by a socio-structural basis.

In this sense, the inventories (breeding males and females, piglets, etc.) and income of backyard livestock production provide key information to determine the economic sustainability of the producers. However, based on the vital importance of the structural element, we were able to accurately determine the sources from which those resources arise. In this study, we were able to describe some of the socio-productive relationships established between the different actors participating in these input and information management mechanisms. The relationships were: 1) input supplier-producer; 2) piglet breeder-swineherd-butcher; 3) religious festivities-pigs; 4) season of the year-pig; and 5) producer-boar keeper. These relationships are imperceptible to the naked eye, but they are part of a series of strategies that allow the reproduction of the livestock system in question. Hence the importance of a continuous analysis of these social components.

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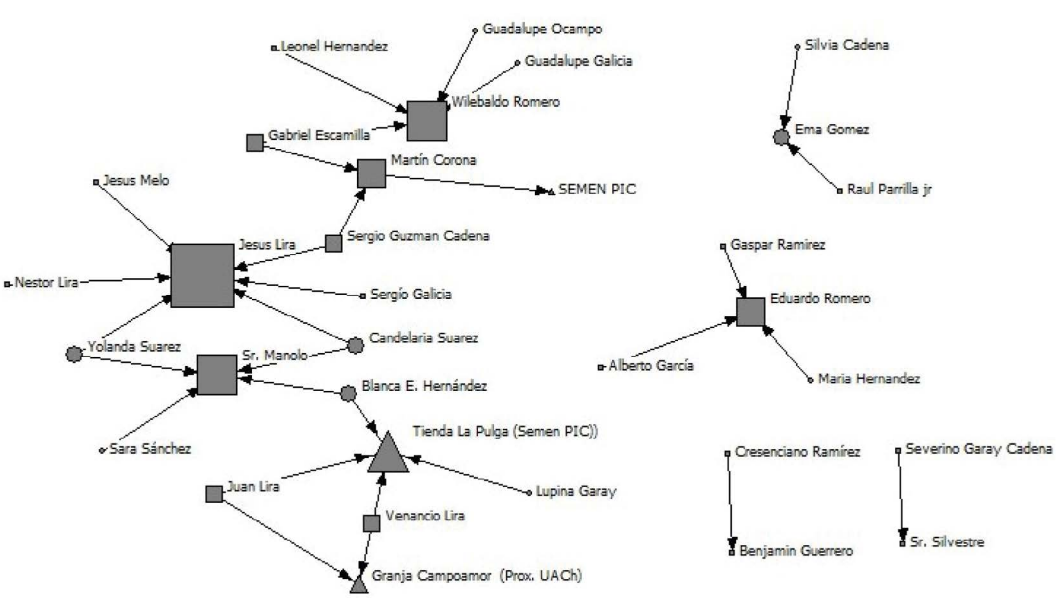

Figure 5. Local boar keepers structure degree in San Miguel Coatlinchán, Texcoco, Mexico.

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AP AGRO PRODUCTIVIDAD

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Determinants of artificial insemination use in cattle in northern Sinaloa, Mexico

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ABSTRACT

Objective: To identify the factors that determine the use of artificial insemination by cattle producers in northern Sinaloa.

Design/methodology/approach: We used a discrete-choice logit model for 200 cattle producers from northern Sinaloa.

Results: Four variables were significant ($p \leq 0.05$): two of them reduce the probability that the farmer will adopt the artificial insemination technology (age and distance from the ranch to the municipality), while the other two increase the probability of adoption (income from the sale of calves and machinery and equipment index).

Limitations/implications: The results only apply to the sample from three municipalities in northern Sinaloa, although they may be useful for other regions with similar characteristics.

Findings/conclusions: Farmers with a higher rate of machinery and equipment have a 36.43% probability of adoption.

Keywords: dry tropics, genetic improvement, technological adoption.

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INTRODUCTION

Animal production systems in tropical areas can contribute to a greater demand for food of animal origin; these systems have the best conditions to increase food production, based on their ability to generate biomass (Chará *et al.*, 2015). In tropical areas, livestock is characterized by the production of milk or cheese and animals for slaughter (Urdaneta, 2019); consequently, this dual-purpose cattle production system is called "Sistemas Bovinos de Doble Propósito (SBDP)". In Mexico, SBDPs are associated with a tropical vegetation

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area of 28.3 million hectares (INEGI, 2019) and a 13% contribution to the national milk production (SIAP, 2020).

The productivity of tropical livestock herds in Mexico is below 50% of their biological capacity (REDGATRO, 2018). In other words, this type of livestock —due to its current conditions— has a high productive potential. Therefore, the improvement of reproductive efficiency is an important step to achieve this potential and to maximize the profitability of the said system (Horrach *et al.*, 2020).

Artificial insemination (IA) is an assisted reproductive technology that generates an accelerated genetic gain that affects the productive, reproductive, and collective performance of the cattle herd, potentially increasing the beef and dairy production (Kubkomawa, 2018; Baruselli *et al.*, 2018). Despite these benefits, few farmers have used and adopted this technology.

In Mexico and other countries, 4-15% of the farmers use this technology (Kubkomawa, 2018; Baruselli *et al.*, 2019; Lassala *et al.*, 2020). In the state of Sinaloa, "livestock is based on undefined crossbreds and it is not focused on productive improvement, which results in low-weight calves at birth, low fattening performance, and low-quality carcasses" (Casillas, 2012, p. 8). Faced with this problem, for more than 20 years, local research centers have implemented projects to promote the use of various technologies (including IA), in the dual-purpose cattle system prevalent in the state of Sinaloa (Loaiza, 2011).

Some studies have used diagnoses to identify the percentage of producers that use IA in the tropical regions of Mexico (Basurto *et al.*, 1997; Cuevas *et al.*, 2013; Rangel *et al.*, 2017, 2020). However, in general there have not been many studies that identify the factors that determine the use of IA; specifically, there are no studies of this type in northern Sinaloa. Perhaps the low use of this technology in the study region can be explained by the excessive (economic, technical, and human) requirements and inputs (such as nitrogen). The objective of this work is to identify the factors that determine the use of artificial insemination by cattle producers in northern Sinaloa.

MATERIALS AND METHODS

Location of the study area

The study was carried out in the state of Sinaloa in northwestern Mexico. Sinaloa is located between the following geographic coordinates: 27° 02' 32 " N, 22° 28' 02" S, 105° 23' 32" E, and 109° 26' 52" W. The weather in the state is divided as follows: 37.14% has a warm sub-humid climate with rainfall in summer A(w); 21.27% has a semi-arid climate that is very hot and hot BS1 (h'); and 18.56% has an arid climate that is very hot and hot BS0 (h'). In the upper parts of the Sierra Madre Occidental (2.26%), Sinaloa has a temperate sub-humid climate with rainfall in summer (INEGI, 2017).

Instrument used

The information was obtained through a survey aimed at the producers who participated in the Proyecto Integral de Innovación y Extensionismo Rural (PIIEX): "Bovinos doble propósito". The project was financed by the Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (SAGARPA). The survey was carried out from

September to November 2015. Nevertheless, the analysis of the variables that determine the use of IA in dual-purpose cattle is still valid, because there is no information on the subject for the region.

A sample of n=200 producers was obtained through non-probabilistic sampling in three municipalities in the north of Sinaloa (Ahome, El Fuerte, and Guasave). The following criteria were used to select the producers: 1) the producer owned cattle; 2) the producer had not been a beneficiary of government programs in previous years; and 3) the producer agreed to answer the survey. The information we obtained included the age, educational level, and number of children of the producer, as well as farm-related aspects (cattle herd and constructions, as well as machines and equipment). The information also covered commercialization, context variables, rural extension services, and distance from the productive unit to the municipality, among others. Cuevas-Reyes and Rosales-Nieto (2018) further described the calculation of the infrastructure, machinery and equipment index.

Econometric model and variables

A discrete-choice logit model was used to estimate the factors that determine the use of IA. These models use a binary categorical variable with two mutually exclusive categories — which can be expressed by numerical values of 1 and 0 (Greene, 2003). The model assumes that there is a Y_i variable that measures the use of the IA by the *i*-th farmer expressed in the following formula: $Y_i = X_i'\theta + U_i$. Where X_i' is a vector of the explanatory variables, θ is an unknown parameter that must be estimated, and U_i is the stochastic term. In this model, the Y_i dependent variable is binary and has two values: $Y_i=1$ (if the farmer uses IA) and $Y_i=0$ (otherwise). Therefore, the binary logistic regression model is given by the following equation (Cameron *et al.*, 2005):

$$p_i = Pr\left[y_i = 1 \middle| x_i\right] = \frac{\exp\left(x_i\beta_i\right)}{1+\exp\left(x_i\beta_i\right)} \tag{1}$$

This ensures that the probability is between 0 and 1. The logit model marginal effects are given by the following equation, that is commonly known as the probability density function of the logistic distribution, multiplied by the coefficient of the explanatory variable under consideration (Cameron *et al.*, 2005):

$$\frac{dp_i}{dx_i} = \frac{\exp\left(x_i\beta_i\right)}{\left(1+\exp\left(x_i\beta_i\right)\right)^2}\beta_i \tag{2}$$

The explanatory variables used for the empirical analysis included information regarding four levels that are recognized in literature as determinants for the adoption of technology: producer, ranch, market, and context variables (Teaklewold *et al.*, 2013; Cuevas *et al.*, 2013; Zuwarimwe and Mbaai, 2015). Two variables are associated with the producer: age (EDAD) and schooling (ESCOLARIDAD). The variables related to the ranch were: productivity measured in liters per cow per day (LITROS/VACA), number of animal units (UA), infrastructure (INFRAE), and machinery and equipment (MAQ). Economic or market variables such as income obtained from the sale of milk (LECHE) and income from the sale of calves (CARNE) were also included. In addition, context variables such as the distance from the ranch to the municipality (DIST) and the number of years with technical assistance (AT) that the producer has had were included. Therefore, the estimated empirical model was the following:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_6 X_6 + \beta_7 X_7 + \beta_8 X_8 + \beta_9 X_9 + \beta_{10} X_{10} + u_i \quad (3)$$

Where: Y=binary variable for the use of IA, β_i=coefficients to be estimated, X_1=EDAD; X_2=ESCOL; X_3=LITROS/VACA; X_4=UA; X_5=INFRAE; X_6=MAQ; X_7=LECHE; X_8=CARNE; X_9=DIST; X_{10}=AT, and u_i=stochastic error.

The information was captured in Excel spreadsheets; subsequently, the information was subject to a statistical analysis using the STATA program (StataCorp, 2015).

RESULTS AND DISCUSSION

Production units and significance of the model

We interviewed producers that had an interest in the extension program: producers with extensive livestock production experience (the oldest producer interviewed was 85 years old), as well as young producers (21 years old) participated in the survey. In general, having a wide variability of participating producers is reflected in the descriptive statistics of the sample (Table 1).

In the producer-related variables, the average age identified was 50.94±13.84 years, while the average schooling was 6.63±5.10 years. It is important to point out that the

Table 1. Characterization of the production units analyzed.

Variable	Mean±SD	Minimum	Maximum
Average age of Producers (years)	50.94±13.84	21	85
Producer schooling years	6.63±5.10	0	16
Average milk per cow	2.63±2.07	0	11.5
Animal units (AU) per ranch	25.87±20.98	4	194.3
Infrastructure index (%)	0.05±0.06	0	0.36
Machinery and equipment index (%)	0.09±0.11	0	0.54
Sale of milk per week ($)	167.50±179.80	0	1250
Sale of calves per year ($)	28468.00±34042.00	0	176000
Distance from the ranch to the municipality (km)	3.06±5.12	0	40
Number of years with technical assistance	1.44±2.33	0	13

participants included producers with undergraduate and even postgraduate studies, as well as producers who did not have any schooling. In the characteristics related to the production unit or ranches, a low average milk production (2.63±2.07) was identified, as well as an average womb inventory level (25.87±20.98) of animal units per ranch. With regard to the variables indirectly related to the capitalization level, very low infrastructure (0.05±0.06) and machinery and equipment (0.09±0.11) indexes were detected. In market-related variables, the income obtained from the sale of milk per week ($167.50±$179.80 MXN) was analyzed, as well as the income obtained from the sale of calves per year, which amounts to $28,468.00±$34,042.00 MXN. This seems to indicate that selling calves has preference over the production of milk. With regard to the context variables —those that do not depend on the production unit—, we recorded the distance in kilometers between the ranch and the municipal seat, as well as the years the producers had received technical assistance. Regarding this variable, is important to point out that the minimum value was 0 years and the maximum value was 13 years. On average, the distance from the ranches is 3.06±5.12 km, while producers have had 1.44±2.33 years of technical assistance (Table 1).

In the sample that we analyzed, only 4% of the farmers used IA technology. The X^2 test was used to evaluate the global significance of the model; the null hypothesis was that all the coefficients of the equation (except for the constant) are null. The number of correctly predicted cases was 97.46%, the LR chi^2 (10) statistic was 46.87, and the associated probability was <0.000; consequently, the null hypothesis is rejected and the global model is proved to be significant. The statistical significance of the estimated parameters was obtained through the Wald test (z-statistic). In the study, the IA was independent ($p>0.05$) of the schooling level and milk production per cow variables, as well of the income from milk sales and the average number of years that the producer had received technical assistance. In contrast, four variables were found to be significant ($p\leq0.05$): age and distance from the ranch to the municipality have a negative influence in the decision to use IA, while the machinery and equipment index, as well as the sale of calves, have a positive influence in the decision of farmers to use this technology (Table 2).

The adoption of IA in the study region is negatively influenced by the age of the producer: older producers are 0.29% less likely to use IA technology. Meanwhile, the machinery index had a significant and positive conditional marginal effect ($p\leq0.05$); this result indicates that producers who had a higher level of machinery and equipment have a probability of adopting the IA of up to 36.43%. In the same sense, the sale of calves was also significant and positive ($p\leq0.05$), which showed that producers who sell calves are 0.00007% likely to adopt IA.

The coefficient of the distance variable was negative and significant ($p\leq0.05$). Consequently, ranches located at a greater distance from the municipality are 2.3% less likely to adopt artificial insemination.

The objective of this work was to identify factors associated with the producer, production unit, income from product sales, and other context variables that determine the use of IA by cattle producers in northern Sinaloa. The results indicate that the IA was independent of variables related to the level of education, milk production per cow, income from milk

Table 2. Coefficients of the variables that influence the probability of using artificial insemination.

Variable	Coefficient	z	P>z	dy/dx
Age	−0.192	−2.04	0.042*	−0.0029
Producer schooling years	−0.281	−0.18	0.853	−0.0005
Average milk per cow	0.373	1.43	0.153	0.005
Animal units (AU) per ranch	0.020	0.84	0.402	.0003
Infrastructure index (%)	−5.842	−0.58	0.564	−0.089
Machinery and equipment index (%)	23.886	2.41	0.016*	0.3643
Sale of milk per week ($)	−0.0007	−0.31	0.756	−0.00001
Sale of calves per year ($)	0.00004	0.043	0.043*	0.00000007
Distance from the ranch to the municipality (km)	−1.553	2.03	0.023*	−0.023
Number of years with technical assistance	−0.407	−0.85	0.396	−0.0005
Constant	−0.281	−0.18	0.816	

dy/dx is the marginal effect of the X variable on the dependent Y variable; dy/dx significance level: $p<0.05$ *. Number of observations (n): 200. LR chi^2 (10)=46.87; Prob> chi^2=0.0000; Pseudo R^2=0.7003, correctly classified=97.46%.

sales, and the average number of years that the producer has had technical assistance. However, the age of the producer and the distance from the ranch to the municipality had a negative impact on the decision to use IA, while the machinery and equipment index and the sale of calves positively influenced the decision of farmers to use this technology. These results are discussed below.

The results obtained indicate that the distance from the production unit or the ranch is a determining factor for the use of IA and, the further away the production unit is from the municipality, the producer is less likely to adopt IA technology. Our results match the findings of Sirajuddin *et al.* (2018), who determined that location is important in the IA process, since the closer the ranch is to the inseminator, the greater the adoption of this practice. Not only are technical personnel more readily available when the producers are closer to the municipal seats, it is also possible to get supplies more quickly (pipettes, gloves, nitrogen, etc.). In other words, it seems that the use of IA is indeed related to a greater availability of the resources that can meet the requirements of this technology.

Additionally, the results indicate that the machinery and equipment index and the sale of calves are preponderant factors that contribute significantly to the adoption of IA. The use and adoption of IA has indeed many requirements, particularly knowledge/experience, personnel, and financial resources for the acquisition of inputs. Our results agree with reports that have indicated that the economic or purchasing factor plays a crucial role in the producer's decision to whether or not use IA (Howley *et al.*, 2012; Cortés-Mora *et al.*, 2014, Rathod *et al.*, 2017; Lassala *et al.*, 2020). It is also evident that age plays an important role in the adoption of technology; since older producers showed little interest in using or learning about IA technology. Therefore; the probability of adopting IA decreases inversely with the age of the producer.

The successful use of the IA technique requires that several factors occur simultaneously. Our results identify factors related to the producer and the productive unit (age, availability of machinery, sale of calves, distance from the ranch to the municipality) as determining factors in the use of this technology. These results complement previous reports that indicate that environmental factors, climate change, inadequate extension services, training, and limited financing reduce the use of IA in production units (Baruselli *et al.*, 2019; Moreki *et al.*, 2019). Taking into consideration the physiology of cows and the insemination technique is also necessary. Therefore, a successful heat detection, the technique used at the time of insemination, semen management (thaw and apply), the metabolic status, and the well-being of the cow are factors that can limit the success of IA (Yehalaw *et al.*, 2018).

The animal units (UA) median for the interviewed producers was 21.6, which indicates —according to Cuevas *et al.* (2016)— that most of the producers analyzed belong to the small-scale producer stratum. This type of producer has a low level of resources, which undoubtedly limits the use of artificial insemination. The results match the findings of Cortés-Mora *et al.* (2012), Cuevas *et al.* (2013), Rangel *et al.* (2017, 2020) who indicate that there is a low technological level in the use of reproduction methods —such as artificial insemination or embryo transfer— in Latin America and that producers with greater purchasing or economic power are more likely to use IA, as reported in Latin America and Mexico by us and other authors (González-Quintero *et al.*, 2020; Lassala *et al.*, 2020).

Sectoral agricultural policies must be aligned with the needs of producers, in order to make production more efficient and to meet the demands for agricultural food production.

CONCLUSIONS

The age of the producer and the distance from the ranch to the municipal seat are variables that have a negative impact on the use of artificial insemination technology; in contrast, income from the sale of calves and the machinery and equipment index have a positive impact on the adoption of artificial insemination by producers from northern Sinaloa. Consequently, producers with a higher level of machinery and equipment have a probability of adopting IA of up to 36.43%. Artificial insemination in the study region requires the definition of a technology diffusion and transfer policy that provides the different producers with permanent information about this technology, availability of inputs, and development of capacities. Only in this sense, the gaps in the use of this technology between small and large producers could be closed, thereby contributing to the improvement of livestock among the producers from northern Sinaloa.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of extraction pH and temperature on the physicochemical properties and pectin yield from mango peel (*Mangifera indica* L.)

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ABSTRACT

Objective: To evaluate the effect of the pH and extraction temperature on the pectin yield from mango (*Mangifera indica*) peel, cultivar Banilejo, and its physicochemical properties.

Design/methodology/approach: Pectin extraction was done by acid hydrolysis, using hydrochloric acid and ethyl alcohol to precipitate and purify. A randomized design with a factorial arrangement was used, evaluating the effect of pH (1.5, 2.0, and 2.5) and temperature (70, 80, and 90 °C) on the yield and quality of pectin. Quality was determined by measuring pH, viscosity and moisture content, ash, methoxyls, and esterification degree. Their means were compared using Tukey's test at 95% confidence.

Results: The best results were obtained at pH 2.0 and 80 °C, reporting an 18.159% yield, 6.766% moisture, 2.630% ash, 0.085 Pa.s of viscosity, 26.307% methoxyl, and 64.753% esterification.

Study limitations/implications: The different treatment combinations demonstrate that pH, ash, methoxyl content, and esterification degree vary as a function of the assessed pH and extraction temperatures; while viscosity, moisture and yield were not influenced by these variables.

Findings/conclusions: It is concluded that mango peel is a viable source to obtain quality pectin.

Keywords: Pectin, *Mangifera indica*, pH, extraction temperature, peel.

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INTRODUCTION

The Dominican Republic stands out for its geo-climatic conditions, suitable for fruit trees production. According to the FAO (2020a) mango continues to be one of the most traded tropical fruits worldwide and in 2019, along with guava and mangosteen, accounting for over a quarter of the total world tropical fruit trade. By 2029, global mango, mangosteens, and guavas production is expected to reach 72.8 million tons, annually increasing by 2.9% over the next decade (FAO, 2020b).

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Mango industrialization residues can be utilized. The peel accounts for 7 to 24% (wet basis) of the total weight of these fruits, according to the variety. Most of the peels are disposed of or used for animal feed. However, these constitute a potential source of pectins and polyphenols with market value and are therefore, exploitable (López, Sañudo, Aguilar, Rodríguez and Contreras, 2011; cited by Martínez, 2018).

Pectins are heterogeneous polysaccharides capable of absorbing large amounts of water and gelling, found in most vegetables and fruits, and in greater proportion in peels; specifically, in the primary cell walls and the middle lamella (Chasquibol, Arroyo and Morales, 2008; Picot-Allain, Ramasawmy, and Emmambux, 2020). Pectins production from fruits waste constitutes a reasonable and promising strategy to obtain natural biopolymers while contributing to recycling and promoting sustainable development (Dranca and Oroian, 2018).

There are different techniques for pectins extraction from plant tissues, either using physicochemical or enzymatic procedures. Their yield depends on the operating conditions, such as temperature extraction, extraction time, pH extraction (Pagan and Ibarz, 1999), extraction solvents, and adding chelating agents that help to release pectins from cell walls (Aldana, Aguilar, Contreras, Villaruel, Nevárez, 2011). According to Púa, Barreto, Vallejo, and Ariza (2015), the most influential factor for pectins extraction is temperature, followed by time, whose variations considerably alter yield.

Therefore, the objective of this research was to evaluate the effect of pH and temperature extraction on the physicochemical properties and yield of pectins obtained from mango (*Mangifera indica* L.) peel, cultivar Banilejo.

MATERIALS AND METHODS

This research was conducted at ISA University, Santiago de los Caballeros, Dominican Republic.

Raw material

The main raw material was mango peels, cultivar Banilejo, acquired from local suppliers.

Experimental design

A completely randomized design, in a factorial arrangement, was used to evaluate the effect of pH (1.5, 2.0, and 2.5) and temperature (70, 80, and 90 °C) extraction on the yield and physicochemical properties of pectins. Nine treatments and three replicates were analyzed, a total of 27 experimental units.

Description of the operations

For the extraction of pectin, the methodology described by Cabarcas, Guerra, and Henao (2012) was followed and included the improvement recommendations stated at the end of their research. Mango peels were weighed, washed, and dried in a Quincy Lab Model 30 convection oven at 60 °C to a 10% moisture maximum. They were then crushed in an HC-500 multifunctional grain mill and sieved on a U.S.A. Standard Test Sieve ASTM E-11 106 μm. Five parts of acidulated water, prepared with 37% pure hydrochloric acid, were added to adjust the pH levels. The acid hydrolysis process was then initiated by placing the treatments in a Thermo Scientific model 2835 water bath with manual agitation, for 20 minutes. The resulting material was filtered through a cloth strainer and manually squeezed (pressing through the strainer) to separate the liquid from the solid material. The pectic solution was rapidly cooled (<25 °C to minimize pectins degradation); then 95 % ethanol, corresponding to twice its volume, was added to induce pectin precipitation. It was left to stand for 60 minutes, then filtered and washed with 50% ethanol to eliminate the chlorides present. It was then placed in the drying oven at 60 °C until constant weight. The dried pectin was crushed, sieved, and analyzed.

Variables evaluated

The variables were analyzed at the Food Safety and Industrial Analysis Laboratory (LIAAI) at ISA University. These were: pH (AOAC 943.02, using a Consort R735 pH meter); viscosity (ISI, 2002), assessed with a Brookfield DV2T Viscometer, at 25 °C and 10 RPM and reported in Pa.s); moisture (AOAC 925.09); ash (AOAC 923.03); methoxyl content (Gierschner, 1997); esterification degree (Owens and McCready, 1952); yield: the final weight of the pectin was related with the initial weight of the peel, following the formula:

$$Yield\left(DB\right)=\frac{Pectins\ weight\times 100}{Mango\ \ peels\ weight}$$

Statistical analysis

The data obtained were evaluated by an analysis of variance (one-way ANOVA). Means were separated using Tukey's test ($P<0.05$). Statistix version 8.0 software was employed and the arithmetic means ± standard deviation was used to present the results.

RESULTS AND DISCUSSION

The results of this research are summarized in Tables 1 and 2. Regard the pH of acidified water, the pH of the obtained pectin from mango peel ranged from 3.442 to 3.814, showing significant differences between treatments means (Table 1). Ferreira, Peralta, and Rodríguez (1995) reported the same pattern, where an increase in the pH of pectin was found when using higher pH solutions, which could be because alkalinity is inversely proportional to the level of hydrogen ions: as pH levels increase, H^+ ions decrease, resulting in higher pH (Cabarcas *et al.*, 2012).

The studied temperature variations (70, 80, 90 °C) produced pH from 3.631 to 3.673, with no difference between the treatments means (Table 1). Paredes, Hernández, and Canizares (2015), in their research about pectin extraction from guava hulls at different maturity stages, at 90 and 95 °C, obtained similar values to reported here, corresponding to 4.05-4.25 (green hulls), 3.8-4.37 (pinto hulls) and 3.9-4.1 (ripe hulls), with no significant differences in relation to the assessed extraction temperatures.

The results of pH on the interaction between extraction pH and extraction temperature are shown in Table 2, with values ranging from 3.41 to 3.83, with the treatments being

Table 1. Effect of pH and temperature extraction on the yield and physicochemical properties of pectins from mango (*Mangifera indica* L.) peel cultivar Banilejo.

		pH	**Viscosity (Pa.s)**	**Humidity (%)**	**Ashes (%)**	**Methoxyls (%)**	**Esterification (%)**	**Pectin yield (%)**
pH	1.5	3.442±0.049 c	0.069±0.008 b	6.768±0.681 a	2.880± 0.125a	26.536±0.243 a	66.897±2.235 b	16.188±1.736a
	2.0	3.706±0.046 b	0.080±0.012 ab	6.808±0.389 a	2.744±0.272a	26.434±0.292 a	67.141±2.628 b	17.206±1.131a
	2.5	3.814±0.047 a	0.083±0.013 a	6.574±0.961 a	2.883±0.161a	26.040±0.111 b	70.241±1.160 a	16.142±1.549a
Temperature (°C)	70	3.673±0.148 a	0.073±0.009 a	6.623±0.522 a	3.002±0.194a	26.432±0.306 a	69.012±2.138 a	16.642±1.404 a
	80	3.631±0.171 a	0.080±0.008 a	6.529±0.399 a	2.751±0.132 b	26.226±0.195 a	66.738±2.617 b	16.739±1.382 a
	90	3.658±0.193 a	0.080±0.018 a	6.993±1.003 a	2.754±0.167 b	26.352±0.394 a	68.529±2.534 ab	16.155±1.845 a

Values placed after the±symbol indicate the standard deviation between the means. Different letters in the same column indicate difference ($P<0.05$) between the means of the evaluated treatments.

Table 2. Effect of the interaction of pH and temperature extraction on the yield and physicochemical properties of pectins from mango (*Mangifera indica* L.) peel cultivar Banilejo.

pH	**Temperature (°C)**	**pH**	**Viscosity (Pa.s)**	**Humidity (%)**	**Ashes (%)**	**Methoxyls (%)**	**Esterification (%)**	**Pectin yield (%)**
1.5	70	3.490±0.062 c	0.072±0.012 a	6.452±0.382 a	2.913±0.158abc	26.493±0.313 ab	68.980±2.304 ab	16.559±0.982 a
	80	3.427±0.021 c	0.073±0.003 a	6.677±0.446 a	2.850±0.173abc	26.370±0.092 ab	66.030±2.018 ab	16.128±1.366 a
	90	3.410±0.010 c	0.062±0.002 a	7.160±1.058 a	2.877±0.063abc	26.743±0.144 a	65.680±0.858 b	15.877±2.976 a
2.0	70	3.723±0.045 ab	0.071±0.004 a	7.180±0.127 a	3.043±0.273ab	26.640±0.305 a	67.247±1.519 ab	16.371±0.431 a
	80	3.667±0.051 b	0.085±0.005 a	6.766±0.269 a	2.630±0.035bc	26.307±0.131 ab	64.753±1.799 b	18.159±0.571 a
	90	3.727±0.021 ab	0.085±0.020 a	6.477±0.374 a	2.560±0.121c	26.357±0.367 ab	69.423±2.387 ab	17.086±1.476 a
2.5	70	3.807±0.032 a	0.075±0.014 a	6.237±0.443 a	3.050±0.180a	26.163±0.065 ab	70.810±1.065 a	16.996±2.535 a
	80	3.800±0.078 a	0.081±0.010 a	6.142±0.159 a	2.773±0.029abc	26.000±0.092 b	69.430±1.595 ab	15.930±0.935 a
	90	3.837±0.021 a	0.091±0.013 a	7.341±1.464 a	2.827±0.078abc	25.957±0.032 b	70.483±0.383 a	15.501±0.719 a

Values placed after the ± symbol indicate the standard deviation between the means. Different letters in the same column indicate difference ($P<0.05$) between the means of the evaluated treatments.

statistically different. The highest value (3.83) is above that reported by Beltrán, Díaz and Sáenz (2011), for commercial pectin derived from citrus products (3.5).

As for the viscosity of mango peel pectins, it is affected by variation pH extraction levels, reporting values between 0.069 and 0.082 Pa.s. This shows a clear tendency to decrease as pH is lowered, which is consistent with the research by Alfonso (2010) about the rheological behavior of pectins, where treatments at pH 1.2 and 70 °C resulted in 0.026-0.032 Pa.s, while treatments with higher pH showed higher values.

The viscosity of mango peel pectin was not affected by the variation of the studied temperatures nor with the pH-temperature interaction, so these results will not be discussed.

Tables 1 and 2 show that the independent factors and their interaction do not influence the moisture content of mango peel pectins. Pectin is a substance with a high-water retention capacity, so moisture content is an important parameter for its commercialization, whose maximum limit is officially set at 12% (Food Chemical Codex, 2003). The moisture results in the present research are within the established limits and are similar to those obtained by Ferreira (2007) in pectin from tropical fruits (1.10 to 5.63%); Cabarcas *et al.* (2012) in pectin from banana peels (1-12%) and 11.04 and 2.10% by Vásquez *et al.* (2008), when using pH 2 and 3, respectively.

Another evaluated response variable was the ash content of the pectin. Table 1 shows that, by modifying the pH of the solution, pectin with 2.74 to 2.88% ash was obtained, with no significant differences between their means. These values are similar to those reported by Normah and Hasnah (2000) and Corona, Díaz, Páez, Ferrer, Mármol and Ramones (2012), 3.40% in kiwifruit and 2.04% in parchita peel, respectively.

The extraction temperature influenced the ash content of the studied pectin, between 2.75% to 3.00%. These results differ from those reported by Lliuyacc (2018) in pectin from Serrano tumbo peel, where despite finding no significant differences in the assessed temperatures (60 °C, 64 °C, 70 °C, 76 °C, and 80 °C), found the highest ash content between 76 °C to 80 °C, the lowest at 60 °C.

The interaction between pH and extraction temperature (Table 2) also affected the ash content of pectin, with values from 2.56 to 3.05%; being similar to those obtained by Cabarcas *et al.* (2012) in banana peel pectin (0.9-3.5%) and different from those reported by Lliuyacc (2018), which range from 5.755 to 23.772%.

Methoxyl values were affected by the extraction pH, ranging from 26.04% to 26.54%. It was observed that the lower the pH, the higher the methoxyl content, which differs from the findings of Vásquez *et al.* (2008), where methoxyl content decreased with decreasing pH, showing 1.47% at pH 2.0 (66% lower compared to that obtained at pH 3.0). These data are consistent with the findings of Pagan and Ibarz (1999), who observed that high temperatures and low pH seem to favor pectin demethylation.

The studied temperature levels resulted in pectins with values between 26.226 and 26.432% of methoxyl, no significant differences between the means are observed. As the extraction temperature increases, the methoxyl content decreases. Lliuyacc (2018) indicates that this may be because increasing the time and extraction temperature increases yield, but the methoxyls percentage reduces due to increased esters hydrolysis in the methoxylated carboxyl groups and directly relates to the pectins quality.

By evaluating the interaction between pH and extraction temperature, methoxyl values ranging from 25.957 to 26.743% were found (in the pH 1.5 treatment at 90 °C), showing statistical differences. These findings are concurring with those by Alfonso (2010), who showed that the temperature-pH interaction produces a higher percentage when the temperature is at maximum and pH at minimum. The results in this research were superior to those obtained by Cabarcas *et al.* (2012) and Vásquez *et al.* (2008) in banana peel pectin and to those obtained by Ferreira (2007) in tropical fruit pectins, being these of low methoxyl in their respective research.

Table 1 also presents the effect of the pH of the solution on the pectin's esterification degree (ED), where values from 66.897 to 70.241% were observed, with a significant difference between the evaluated means. These results show that increasing the pH produces an increase in ED, which is consistent with the values obtained by Flores, Mariños, Rodríguez, and Rodríguez (2014), who, by increasing the pH from 2 to 3, obtained a 53.8 to 65% increase in ED. They also concur with the data obtained by Rodríguez and Rodríguez (2018) in their research on the concentration of citric acid in the quality of pectin's, where, when using 0.2% obtained 86.51% ED, while when using high concentrations, the results were lower.

The ED of mango peel pectins is also affected by the extraction temperature, resulting in values between 66.738 and 69.012%. The pectin with the highest ED was the one extracted at 70 °C, probably because moderate temperatures allow reaching higher esterification degrees.

The results of the ED in relation to the pH-extraction temperature interaction are shown in Table 2, with values ranging from 64.75 to 70.81%, the treatments being statistically different. These data are lower than those reported by Ferreira (2007) for tropical fruit pectins (84.2-93.5%). Except for the treatments corresponding to pH 2, by combining low pH values and temperatures, better results are obtained. This is consistent with the research by Flores *et al.* (2014), whereby using pH between 1 and 1.5 and temperature between 70 and 80 °C, the ED was higher than 80%, concluding that low pH and moderate temperatures are needed to achieve high ED, indicating good pectin quality (Baltazar, Carbajal, Baca and Salvador, 2013).

The yield of mango peel pectins was not affected by the pH or extraction temperature (Table 1), nor by the combination of these factors (Table 2). Regard the pH, the values were between 16.142 and 17.206%. There are divergences because of the effect of the extraction pH on the results of the final pectin, since research, such as that of Flores *et al.* (2014) showed a tendency that at constant extraction times, the decrease in pH produces an increase in the yield of extracted pectin. Still, Lliuyacc (2018) reports that the higher the pH, the higher the yield. In the present research, the highest yield was obtained at pH 2.

The evaluated temperatures resulted in yields between 16.155 and 16.739%, without significant changes; being numerically higher the one obtained at 80 °C. This concurs with that reported by Flores *et al.* (2014), in French lemon peel, who obtained the best values at temperatures between 73 and 80 °C. According to Púa *et al.* (2015), the most influential factor in pectin extraction is temperature, since high temperatures increase the hydrolysis of protopectin (insoluble) bonds, converting it into water-soluble pectin.

Regard the interaction between pH and extraction temperature, yields from 15.501 to 18.159% were found. Although the means are statistically equal, the treatment with the highest yield (18.159%) was the one extracted at pH 2.0 and 80 °C. These results are close to the highest levels of commercial pectin extraction, such as that from sunflower, with 25% obtained by Rojas, Perea, and Stashenko (2008), cited by Baltazar *et al.* (2013). Similarly, Cabarcas *et al.* (2012) obtained a maximum yield on a dry basis of 23.06% when extracting pectin from banana peels at pH 1.5 and 80 °C and its minimum yield at a temperature of 73 °C and pH 2.7.

CONCLUSIONS

The results show that pH, ash, methoxyl content, and esterification degree vary according to the applied pH and extraction temperatures, while viscosity, moisture, and yield are not influenced by these variables. The best results were obtained when using the pH*temperature combination of 2.0*80 °C. No difference was observed between the yields of the evaluated treatments, but numerically, the highest yield was obtained when using the pH*temperature combination 2.0*80 °C, which corresponds to the intermediate assessed values.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Biological effectiveness of quillay (*Quillaja saponaria*) extracts for the control of yellow aphids (*Melanaphis sacchari*) in sorghum

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ABSTRACT

Objective: To evaluate and define a plant extract for the control of the yellow aphid in order to prevent environmental contamination and improve crop profitability.

Design/Methodology/Approach: In a sorghum plot —located in the municipality of Jantetelco, Morelos— with a high incidence of yellow aphids, three doses —2-, 4-, and 6-mL L^{-1} of water of *Quillaja saponaria* extract, 0.2-mL L^{-1} of water of Imidacloprid, and 10-mL L^{-1} of water of calcium polysulfide—, as well as an absolute control, were evaluated. The applications were carried out using a 15-L manual spray backpack, which had been previously calibrated and had a fan nozzle. A randomized block experimental design —with six treatments and four repetitions— was used. The experimental unit was made up of five 5-m long rows with 0.70 m between rows, resulting in a 17.5 m^2 total area per experimental unit. The three central furrows were the useful plot, leaving 0.5 m at each end of the furrow. The total size of the experimental plot was 420 m^2.

Results: The biological effectiveness during the samplings was as follows: both the *Quillaja saponaria* (6-, 4- and 2-mL L^{-1} water doses) and Imidacloprid treatments had a 100% effectiveness.

Study limitations/implications: This study was carried out in sorghum crops.

Findings/conclusions: Treatments based on *Quillaja saponaria* extracts on *M. sacchari* in sorghum crops showed 100% biological effectiveness from the first application and no phytotoxicity was observed in any treatment.

Key words: aphids, vegetable extracts, *Sorghum bicolor*, agroecological management.

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INTRODUCTION

The yellow sorghum aphid, *Melanaphis sacchari* (Zehntner, 1897), is a pest that originated in Africa (Singh *et al.*, 2004). It prefers to colonize sorghum and sugarcane (Blackmand and Eastop, 2015); however, many grown and wild poaceae can also act as a host for these aphids (Peña-Martínez *et al.*, 2015). This insect appeared in Mexico in November 2013 (Rodríguez del Bosque and Terán, 2015), feeding from and severely damaging sorghum

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(*Sorghum bicolor*) in northern Tamaulipas (Mexico). These pests attacked sorghum because it is their favorite crop and Johnson Grass (*Sorghum halepense*) is their main alternative host. It is believed that Texas and Louisiana are the geographical origin of this infestation. This pest was apparently brought into Mexico by the movement of the winds and tropical storms and in agricultural machinery and equipment that had been used in infected areas, as well as by winged adults that flew from the United States (SENASICA, 2014).

This pest caused 30-100% losses in Tamaulipas (Rodríguez del Bosque and Terán, 2015). The presence of yellow aphid reduces the quality sorghum forage, especially for cattle, sheep, and goats (Rodríguez del Bosque *et al.*, 2018).

The yellow sorghum aphid appeared for the first time in Morelos in 2015, causing complete disasters with up to 50% yield losses (SIAP, 2018). The low thermic requirements it needs to complete its biological cycle mean that the yellow aphid is the only one of its kind that has a very fast reproductive stage (Setokuchi and Muta, 1993). The damage caused to sorghum by *Melanaphis sacchari* depends on several factors (by themselves or combined), including: population density, duration of the infestation, tolerance or susceptibility of sorghum germplasm (Singh *et al.*, 2004; Aranda-Lara *et al.*, 2019), presence of natural enemies of the yellow aphid —mainly predators, parasitoids, entomopathogenic fungi, virus, and bacteria—, and some abiotic factors —such as temperature, relative humidity, rain, and wind (Gallou *et al.*, 2016). The plant's phenological stage also influences the presence of the insect, although significative infestations appear during the final growth stages of the plant and during drought periods.

The insect causes direct damage to the plant suctioning the sap of its leaves; the leaves then turn brown, and consequently their growth is delayed and the crop performance is affected.

Figure 1 shows sooty mold growing on the foliage of the plants (indirect damage), affecting its photosynthetic capacity. *M. sacchari* can spread viruses such as the *Sugarcane Mosaic Virus* (SCMV), the *Sugarcane Yellow Leaf Virus* (ScYLV), and other diseases (SENASICA, 2014).

Since the appearance of this insect, researchers have been looking at ways to control it. Genetic resistance has been studied; however, no hybrids strong enough to resist it have been found (Aranda-Lara *et al.*, 2019). The most efficient control has been the application of chemical products; however, these products have side effects harmful for humans, animals, and the environment. In order to prevent this situation, bioinsecticides and vegetable extracts have been proposed to fight the infestation. A *Verticillium lecanii*-based

Figure 1. High population of *Melanaphis sacchari* causing direct damage to a *Sorghum bicolor* leave.

bioinsecticide has proved to be lethal to the aphid (SENASICA, 2014). During experiments, cempasúchil (*Tagetes erecta*) and higuerilla or castor oil plant (*Ricinus communis*) extracts had been able to control the nymphs of the yellow aphid (Rodríguez del Bosque *et al.*, 2018). As a result of the promising benefits of bioinsecticides, a proposal to study the effects of quillay or soap bark tree (*Quillaja saponaria*) extracts on the yellow aphid (*Melanaphis sacchari*) and to obtain the most effective dose for the treatment was presented.

MATERIALS AND METHODS

Experimental design

A randomized block experimental design —with six treatments and four repetitions— was used. The experimental unit had five 5-m long furrows with 0.70 m between them, with a 17.5 m^2 total area per experimental unit. The three central furrows were the useful plot, leaving 0.5 m at each end of the furrow. The total size of the experimental plot was 420 m^2.

Treatments

The studied treatments are shown in Table 1. Quillay was chosen because it has saponin, which is a biodegradable non-volatile organic compound that works as fungicide, nematicide, and insecticide and promotes growth (Correa and Martínez, 2013). A 2-6 mL L^{-1} range was evaluated because the dose was not determined yet. A treatment using Imidacloprid was used as commercial control, because it is the most used product in the region. Calcium polysulfide was included because the sorghum producers said that it had been effective during the last sowing. In order to evaluate the biological effectiveness, an absolute control —a treatment in which the insect was not controlled— was included.

Number of applications: Three doses were applied on the foliage, with seven days intervals.

Specification of the application equipment and spraying volume: The applications were carried out using a SWISS MEX manual spray backpack, with a previously calibrated fan nozzle. After the calibration, the spraying mean volume was 15 L for four repetitions.

Supplies used in this study: For this study, Adhefol was used as adhesive; the dose was 0.5 mL L^{-1} of water.

Evaluation method: A quantitative evaluation method was applied, recording the total number of live aphid organisms (nymphs, apterous adults, and winged adults) per

Table 1. Evaluated treatments and doses for the control of yellow aphid on sorghum.

Treatments	Doses	Products
1	2 mL L^{-1}	*Quillaja saponaria* extract
2	4 mL L^{-1}	*Quillaja saponaria* extract
3	6 mL L^{-1}	*Quillaja saponaria* extract
4	10 mL L^{-1}	Calcium polysulfide
5	0.2 mL L^{-1}	Imidacloprid
6	--	Absolut test

4 cm^2 of leaf surface. The means of the counts carried out in four plants per treatment were used. The data obtained was subjected to an analysis of variance, using the SAS ver. 12.1 statistical software (SAS, 2012); given the highly significative difference between treatments, a Tukey test (α=0.05) was carried out to define the best treatments.

The ABBOTT Formula was used to calculate each treatment's biological effectiveness (EB), based on the number of live aphid organisms (Abbott, 1925):

$$EB = (IT - it / IT)100$$

where: *IT*=severity in the untreated control; *it*=severity of the treatment.

Sampling type, size, method, and frequency

A quantitative evaluation was applied, recording the number of live *Melanaphis sacchari* per 4 cm^2 of leaf: total number of nymphs, apterous adults, and winged adults per 4 cm^2/ total number of sampled leaves.

The sample size comprised 16 leaves per treatment (four leaves per experimental unit), randomly collected from the useful plot. One sampling was carried out before the application of the treatments and three samplings were carried out —six days apart from each other— after the applications.

RESULTS AND DISCUSSION

Effect of *Quillaja saponaria* on the number of *M. sacchari* nymphs and adults

Before the treatments were applied, an evaluation was carried out in all experimental sorghum crop units, during which 137.75-157.5 yellow aphid nymphs and adults per 4 cm^2 of leaf area were detected. Based on the statistical analysis, there were no significative statistical differences; therefore, all plots had the same initial conditions.

The aphid count was carried out after the treatments were applied. The results of the analysis of variance indicated that there was a highly significative difference between treatments. When the Tukey test (5% significance) was applied, *Q. saponaria* extract treatments had the best results, while calcium polysulfide had the worst results. *M. sacchari* populations decreased up to 93% when *Quillaja* treatments were applied: the 6 mL L^{-1} water doses had the best numerical results, while the Imidacloprid treatment had a 37.5% effectiveness.

During the second sampling, the statistical analysis and the means comparison once again showed highly significative differences between the *Q. saponaria*, the absolute control, and the calcium polysulfide doses. *Q. saponaria* and Imidacloprid treatments kept aphid populations at a zero level (*i.e.*, they had a 100% biological effectiveness) (Figure 3).

During the third sampling, there was once again a highly significative difference between the *Q. saponaria*, the absolute control, and the polysulfide doses. *Q. saponaria* and Imidacloprid treatments kept aphid populations at a zero level (*i.e.*, they had a 100% biological effectiveness) (Figure 4).

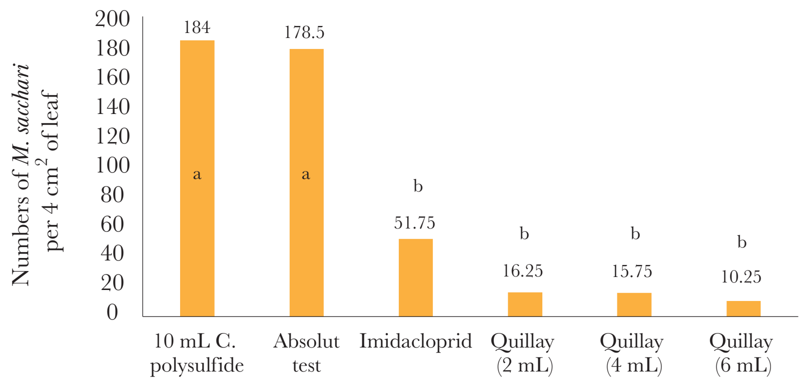

Figure 2. Number of yellow aphid nymphs and adults per 4 cm^2 of leaf area, after the treatments were applied for the first time, in order to evaluate their biological effectiveness. Means with different letters in the columns indicate significant statistical differences between treatments ($P \leq 0.05$).

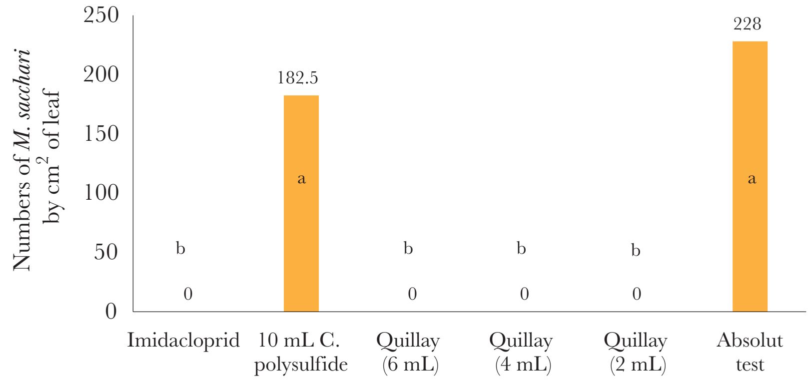

Figure 3. Number of yellow aphid nymphs and adults per 4 cm^2 of leaf area, after the treatments were applied for the second time, in order to evaluate their biological effectiveness. Means with different letters in the columns indicate significant statistical differences between treatments ($P \leq 0.05$).

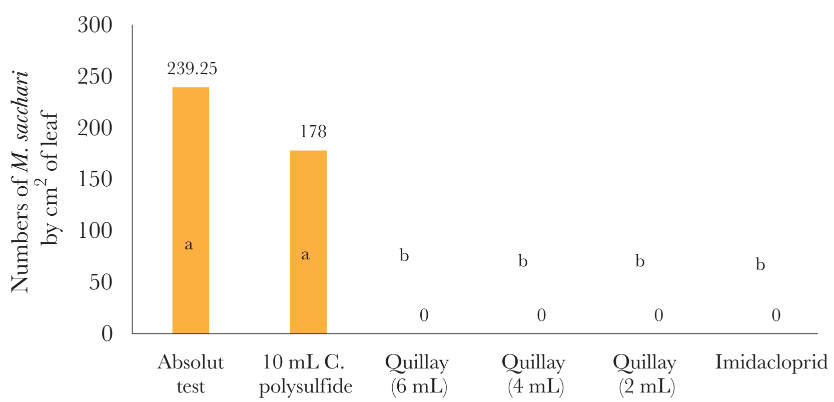

Figure 4. Number of yellow aphid nymphs and adults per 4 cm^2 of leaf area, after the treatments were applied for the third time, in order to evaluate their biological effectiveness. Means with different letters in each column indicate significant statistical differences between treatments ($P \leq 0.05$).

According to the first sampling, the biological effectiveness of the treatments indicates that *Quillaja saponaria* extract treatments, with 6-, 4-, and 2-mL L^{-1} water doses had 93, 89, and 89% effectiveness, respectively, while Imidacloprid had a 62.5% effectiveness.

During the second and third samplings, the *Quillaja saponaria* extract samples, with 6-, 4-, and 2-mL L^{-1} water doses, had a 100% biological effectiveness, while Imidacloprid also had a 100% effectiveness.

Figure 5 shows the biological effectiveness percentage of the treatments evaluated in this study against *Melanaphis sacchari*, after the third aphid count for the third and last application.

Phytotoxicity evaluation

Phytotoxicity was recorded in sorghum plants —particularly in young leaves and their whorls— during the three evaluations; however, the application of the treatments used in this study did not have a phytotoxic effect.

CONCLUSIONS

From the first application, treatments based on *Quillaja saponaria* extracts, with 6-, 4-, and 2-mL L^{-1} water doses, had 93, 89, and 89% effectiveness against the yellow sorghum aphids (*Melanaphis sacchari*), respectively, while Imidacloprid had a 62.5% effectiveness. The third application of the *Quillaja saponaria* doses applied in this study, with 6-, 4-, and 2-mL L^{-1} water, had a 100% biological effectiveness, while Imidacloprid had a 100% effectiveness. None of the treatments used in this study showed any of the phytotoxicity symptoms that affect sorghum crops.

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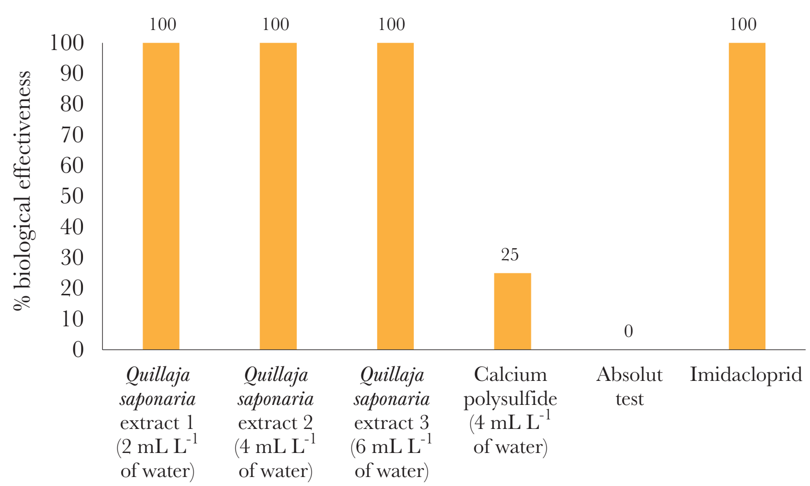

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of maternal intrinsic factors on productivity and efficiency at weaning in commercial flock of Pelibuey ewes in the tropic of Tabasco, Mexico

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ABSTRACT

Objective: To evaluate maternal intrinsic effects (body weight, body condition score, udder type, litter size, lamb sex) on litter growth traits (litter weight at lambing [LBW] and at weaning adjusted for 70 days [LWW70]) as a criteria of productivity and some indexes of efficiency in Pelibuey ewes.

Design/Methodology/Approach: Data of body weight (BW), body condition scores (BCS), and udder types (UT) were taken at lambing, likewise at weaning BW (WBW), on 48 multiparous Pelibuey ewes. Additionally, litter size, sex, birth and weaning weight, were recorded for each offspring; this information was used to calculate LBW, LWW70, average daily gain (ADG), and the following indexes: Ewe efficiency (EEF) expressed as kg of lamb weaned per kg ewe BW at lambing, $EEF^{0.75}$ expressed as kg of lamb weaned per kg ewe metabolic BW ($BW^{0.75}$) at lambing, and EEF2 expressed as kg of lamb weaned ± ewe BW gained or lost at waning per kg ewe BW at lambing. For statistical analysis, ewes were classified according to BW (low, medium and high), BCS (low and high) and udder type (I, II, III and IV) at lambing. High BW ewes had greater ($P \leq 0.01$) LLW and LWW70 than low BW ewes.

Results: Ewe BW at lambing did not affect ($P > 0.05$) any productivity index. Also, BCS from lambed ewes did not alter ($P > 0.05$) the productivity parameters. The udder type affected only LWW70 and productivity indexes, being similar among ewes with type-I, II and III udders, but lower in ewes with type-IV udders. Likewise, double litters were heavier at birth and weaning than single litters, affecting EEF and $EEF^{0.75}$, but not EEF.

Conclusions: The Pelibuey ewe's productivity in humid tropic depends on the maternal BW, udder type at lambing and prolificacy, being a better productivity when ewes have a high BW, double lambing and non-misshapen udders.

Key words: Hair ewes; pre-weaning growth; udder score; lactating sheep.

Image by Here and now, unfortunately, ends my journey on Pixabay at Pixabay

INTRODUCTION

In the tropical regions of Mexico, hair sheep play an important role due to its contribution in the national meat production, and represents a great alternative for tropical agricultural diversification, as they can be raised both in extensive and intensive systems (Chay-Canul *et al.*, 2016). So, the lamb production is one of the main activities in tropical areas of southeastern Mexico, where Pelibuey is the main hair breed used as maternal line due to their prolificacy, low reproductive seasonality, rusticity and high adaptation capacity to extreme climate (Gutierrez *et al.*, 2005; Chay-Canul *et al.*, 2016; Chay-Canul *et al.*, 2019).

Pelibuey sheep are being raised for meat production, and in consequence, farmers are interested in identifying productive traits for selection purpose to improve the ewe productivity. An interesting trait is related to amount of lamb kilograms per ewe at weaning, but without dramatically increasing production costs. In order to improve ewe efficiency or productivity (composites and complex traits), it is important to recognize some intrinsic maternal factors altering the potential to produce heavier litters per ewe lambed (Iñiguez and Hilali 2009; Lôbo *et al.*, 2011). Breed, individual variation between ewes, body size, body condition score, age, mothering ability, milk production, udder morphology, prolificacy, and lamb growth and survival rate are factor associated with the ewe productivity (Fogarty, 1995; Safari *et al.*, 2005). For example, ewes' body weight (BW) is used as a selection criterion between and within breeds because it has a positive correlation with birth and weaning lamb weight (Fogarty, 1995; Segura *et al.*, 1996; Petrović *et al.*, 2012). In addition, ewe BW at lambing has a positive correlation with milk production and, therefore, lamb pre-weaning growth, but with a greater energy maintenance cost (Ángeles *et al.*, 2013; Hinojosa-Cuéllar *et al.*, 2019). However, more information is required on the impact of maternal BW on Pelibuey ewe productivity.

Ewe efficiency can also be evaluated by measuring weaning litter weight (WLW) or through the ratio of WLW: live or metabolic BW of the ewe at lambing (Lôbo *et al.*, 2012; Chay-Canul *et al.*, 2019). Moreover, recently Beard *et al.* (2019) reported that the poor conformation of udder and teats may reduce the profitability of livestock production systems due to decreased offspring weaning weight, increased incidence of mastitis, and decreased productivity lifetime of females. Additionally, udder conformation may negatively affect milk yield available for offspring pre-weaning development (Ugarte and Gabina, 2004; Beard *et al.*, 2019). It should be mentioned that, for hair breed raised in tropical production systems, there is limited information of the effects of udder conformation at parturition on the production of weaning lamb per ewe. For the aforementioned, it was hypothesized that ewe BW and body condition score (BCS), udder type (UT), litter size, and offspring sex affect pre-weaning growth in Pelibuey breed lambs, which in turn modifies productivity and efficiency of the dams. Therefore, the aim of this study was to evaluate the effects of ewe BW, BCS, udder type, litter size and lamb sex at lambing on LWW, average daily gain (ADG) in lambs as a criterion of ewe productivity and efficiency under humid tropic conditions of southeastern Mexico.

MATERIALS AND METHODS

Experimental site, animals, and handling

All animal handling was carried out in compliance with guidelines and regulations for ethical animal experimentation according to División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco. The experiment was carried out at the Sheep Integration Center of the Southeastern (Centro de Integración Ovina del Sureste [CIOS]; 17° 78" N, 92° 96" W; 10 masl), located on the km 25 + 3 of the Villahermosa-Teapa road, in the state of Tabasco, Mexico.

This study was conducted with 48 multiparous Pelibuey ewes, which had at lambing an average BW of 29.8±5.30 kg, BCS of 2.65±0.35 from a 5-point scale (Russell *et al.*, 1969), and age between 2 and 3 years. Ewes grazed during the daylight in paddocks of irrigated and fertilized Star grass (*Cynodon plectostachyus*) and housed overnight. Females had free access to water and mineral salts and were supplemented with 300 g of a commercial feed each day (16% crude protein; AFRC, 1993). Lambs were weighed within the first 24 h post-lambing and subsequently at weaning. During the pre-weaning period, lambs were penned in pens while ewes grazed from 7:00 h to 16:00 h. Dams and lambs were gathered in the same pen during the afternoon and night when they were returning from grazing. Weaning was performed at approximately 70 days of age. Lambs received chopped grass *ad libitum* and around 100 g of a commercial concentrate (16% crude protein) from the first week of age until weaning.

Measurements in ewes and lambs

Data collected included ewe identity, date of lambing, ewe BW, BCS and udder type (UT), which were recorded 24 h after lambing. The BCS was measured using a 5-point scale, where BCS 1 corresponded to a very thin ewe and BCS 5 to a very fat ewe (Rusell *et al.*, 1969; Thompson and Meyer, 1994). The UT was classified based on the position/insertion of teats into four types (Rovai *et al.*, 2004): type I=horizontal teats, type II=teats at 45 degrees; type III=vertical teats (most desirable for "udder machine"), and type IV=misshaped udder. In addition, ewe BW at weaning (BWW) and litter size (LS) at lambing and weaning were recorded. In the case of lamb measurements, identification, birth date and weight, weaning date and weight, sex, lambing litter weight (LBW), WLW, and age at weaning (days) were recorded.

From the information collected on ewes and lambs, both LWW adjusted at 70 days of age (LWW70=LLW+ADG×70 d) and pre-weaning average daily gain (ADG=[LWW−LBW]/age at weaning) were calculated as criteria of ewe productivity. In addition, it was calculated different indexes of ewe productive efficiency (EEF): 1) EEF=WLW/ewe BW at lambing, 2) $EEF^{0.75}$=WLW/ewe $BW^{0.75}$ at lambing, and 3) EEF2=ewe BW gained or lost at waning/ewe BW at lambing.

Statistical analyses

Initially, all study variables were subjected to normality with the Shapiro Wilk test using W statistic. Then, an analysis of variance was performed fitting a lineal model with PROC

GLM of the Statistical Analysis System software, version 9.0 (SAS Institute Inc, Cary, NC, USA). The fixed effects included in the model were ewe BW, BCS, UT and litter type; all factors considering its measurement at lambing. The levels of each factor were as follows: ewe BW=high (>32.0 kg), medium (28.0 to 31.0 kg) and low (<27.2 kg); ewe BCS=high (≥3 points) and low (≤2.5 kg); UT=I, II, III and IV; and little type=M (single lambing with a male), F (single lambing with a female), MM (double lambing with two males), FF (double lambing with two females) and FM (double lambing with male and female). Means were compared using the Tukey-Kramer test at P<0.05.

RESULTS AND DISCUSSION

Ewe BW affected (P<0.05) LBW, ADG and LWW, being the high BW ewes that had the heavy litter at birth and weaning (Table 1). While BCS did not affect (P>0.05) the study variable, UT and litter type modified (P<0.05) ADG, LWW70, and all productive efficiency indexes with exception of EEF2. Ewes with udder classified as type I, II and III had similar (P>0.05) LWW70, ADG and efficiency indexes, but all those variables had the lowest (P<0.05) mean values in ewes with type-IV udder (Table 2). Double litters with any combination of sexes had similar (P>0.05) LLW, ADG, LWW70, EEF and $EEF^{0.75}$. All those variables in F single litters had lower (P<0.05) mean values than in double litters with FM and MM, but similar (P>0.05) compared to M single litters. Additionally, only

Table 1. Least square means and standard errors of lambs pre-weaning growth traits as a criteria of ewe productivity.

	n	LBW (kg)	ADG (kg/Day)	LWW70 (kg)
Ewe body weight		P<0.001	P<0.001	P<0.001
High	17	3.478±0.161[a]	0.170±0.008[a]	15.404±0.622[a]
Medium	16	2.844±0.164[b]	0.138±0.009[b]	12.476±0.632[b]
Low	15	2.410±0.190[b]	0.130±0.008[b]	11.566±0.730[b]
Ewe body condition score		NS	NS	NS
2.5	31	3.055±0.119[a]	0.143±0.005[a]	13.052±0.459[a]
3	17	2.766±0.175[a]	0.150±0.008[a]	12.245±0.672[a]
Udder type		NS	P<0.001	P<0.001
I	8	2.617±0.242[a]	0.171±0.011[a]	14.580±0.933[a]
II	19	2.850±0.201[a]	0.166±0.010[a]	14.445±0.773[a]
III	13	3.221±0.213[a]	0.186±0.010[a]	16.226±0.820[a]
IV	8	2.953±0.387[a]	0.118±0.017[b]	11.25±1.075[b]
Litter Type		P<0.0001	P<0.0001	P<0.0001
F	15	1.963±0.218[a]	0.105±0.011[a]	9.344±0.839[a]
FF	7	3.237±0.239[ab]	0.148±0.012[b]	14.027±0.920[b]
M	12	2.236±0.231[a]	0.123±0.011[ab]	10.848±0.891[ab]
FM	8	3.621±0.328[b]	0.153±0.012[b]	14.152±0.922[b]
MM	6	3.495±0.563[b]	0.202±0.017[b]	17.370±1.167[b]

LWB=Litter weight at birth (kg), LWW70=Litter weight at weaning adjusted at 70 days, ADG: average daily gain of lambs (g/d. Means with different superscript indicated statistical differences (P<0.05).

ADG and WLW70 were different ($P<0.05$) between F single and FF double litters, being higher in the latter.

Knowledge about environmental factors affecting pre-weaning performance of lambs is useful to modify and/or propose feeding and handling strategies in lambs, which could optimize productive efficiency (Oliva-Hernández and García-Osorio, 2016). However, there are few studies related to these factors in hair sheep. Although the current study included a low number of animals, results partially support the hypothesis as ewe BW, UT and litter size modified lamb pre-weaning growth, and this was reflected on ewe productivity and efficiency. The BCS was not a predisposing factor for lamb pre-weaning growth and ewe productive efficiency.

Results of the present study showed that heavier ewes at lambing had 45 and 28% more litter weights at birth and weaning, respectively, compared to low BW ewes. It has been reported that maternal BW is a very important characteristic in animal husbandry, since increases in dam BW improves reproduction efficiency and profitability in ewes (Cam *et al.*, 2010; Aliyari *et al.*, 2012; Benchohra *et al.*, 2015). In Rembi ewes, Benchohra *et al.* (2015) reported that heavier ewes at lambing produced 10.26% more milk than lightweight ewes. This finding is relevant as high daily milk yield is related with high pre-weaning ADG and finally weaning weight in offspring (Ünal *et al.*, 2008; Sezenler *et al.*, 2016). Although milk production was not measured in the current study, results from LWW70 and ADG due to ewe BW at lambing were attributed to this fact. In line with our results, Hinojosa-Cuellar *et al.* (2019) found that the ewe BW at lambing affected the pre-weaning performance in hair lambs raised under tropical production systems, being heavier at birth and weaning lambs born from ewes with high BW at lambing.

With regard to ewe BW on productivity, the trend found in the current study is similar to those reported by Segura-Correa *et al.* (1996), when studying the productivity of Pelibuey and Blackbelly sheep under extensive management conditions in Mexico, reported lower productivity and prolificacy in ewes with lower BW than the average BW of the group. Overall, results of ewe productivity agree with those reported for Katahdin and Pelibuey breed ewes kept in tropical conditions of Mexico (Nasrat *et al.*, 2016; Chay-Canul *et al.*, 2019). On the other hand, with regard to the effect of BCS on the lamb pre-weaning performance, Kenyon *et al.* (2014) reported that ewe BCS has no influence on pre-weaning ADG and weaning weight in lambs. These results are similar to ours. In Romney breed, ewes with a BCS of 3.5-4.0 at breeding had lambed lighter offspring than ewes with a BCS of 3.0. (Kenyon *et al.*, 2004). However, in the study of Cranston *et al.* (2017), the ewe BCS had no effect on lamb birth weight, which is congruent with our results. Sezenler *et al.* (2016) and Cranston *et al.* (2017) suggested that controlling maternal BCS ($BCS>2.0$) in late pregnancy and early lactation may increase milk production and consequently to improve both ewe and lamb performance at weaning.

Several authors have reported that udder characteristics do not belong to the most important traits of the lactating sheep, but they could have strong effects on the milk yield and composition, and consequently lamb growth rate (Ugarte and Gabina 2004; Sezenler *et al.*, 2016). Sezenler *et al.* (2016) stated that the relationships among morphological udder

Table 2. Least square means and standard errors of ewe efficiency.

	n	EEF	$EEF^{0.75}$	EEF2
Ewe body weight		NS	NS	NS
High	17	0.431±0.020[a]	1.055±0.046[a]	0.490±0.031[a]
Medium	16	0.407±0.020[a]	0.958±0.046[a]	0.476±0.037[a]
Low	15	0.448±0.023[a]	1.009±0.054[a]	0.498±0.036[a]
Ewe body condition score		NS	NS	NS
2.5	31	0.438±0.014[a]	1.021±0.034	0.499±0.023
3	17	0.420±0.021[a]	0.994±0.049	0.477±0.034
Udder type		P<0.01	P<0.01	NS
I	8	0.456±0.029[a]	1.081±0.068[a]	0.507±0.046[a]
II	19	0.478±0.024[a]	1.117±0.057[a]	0.517±0.038[a]
III	13	0.517±0.026[a]	1.224±0.060[a]	0.603±0.040[a]
IV	8	0.358±0.039[b]	0.788±0.078[b]	0.428±0.052[a]
Little Type		P<0.001	P<0.001	NS
F	15	0.324±0.026[a]	0.748±0.061[a]	0.385±0.041[a]
FF	7	0.439±0.040[ab]	1.045±0.092[ab]	0.513±0.046[a]
M	12	0.378±0.028[a]	0.873±0.065[a]	0.412±0.045[a]
FM	8	0.454±0.029[b]	1.070±0.067[b]	0.530±0.064[a]
MM	6	0.548±0.048[b]	1.299±0.109[b]	0.596±0.089[a]

EEF: Calculated as (LWW70)/BW of ewe at lambing); $EFE^{0.75}$: Calculated as (LWW70)/ $BW^{0.75}$ of ewe at lambing; EEF2: expressed as kg of lamb weaned ± ewe BW gained or lost at waning per kg ewe BW at lambing. Means with different superscript indicated statistical differences (P<0.05).

traits would permit to predict future correlated responses in milk-oriented selection schemes in sheep. To date, in Pelibuey sheep, few studies have evaluated udder morphological traits and their relationship with milk yield (Arcos-Alvarez *et al.*, 2020; Espinoza-Mendoza *et al.*, 2020) and performance of suckling lambs (Chay-Canul *et al.*, 2021). In the present study, it was observed that lambs had a similar LLW in ewes scored in different udder types. However, lambs from dams with udder scores from I to III had higher ADG by almost 57% compared to lambs from ewes with type-IV udder; this was reflected in 47% more WLW70 for ewes with type-I, II and III udders. With regard to prolificacy, several studies indicated that ewes rearing twin lambs had higher productive efficiency compared with single bearing ewes. Chay-Canul *et al.* (2019) reported that WLW70 and EEF were similar between Katahdin and Pelibuey with single- or twin-bearing ewes. In addition, the authors mentioned that both ewe productivity and efficiency are better for twin-bearing ewes than for single-bearing ewes, regardless of genotype. These results agree with the present study.

Overall, when productive efficiency was evaluated, ewes with double litter (MM and MF) were more productive based on live and metabolic BW compared to ewes with F single litter. However, this difference disappeared when efficiency index was obtained using ewe BW change across the pre-weaning period. These findings suggest that Pelibuey ewes efficiently using energy consumed for meat production, either in the form of weaning lamb kilograms or in the accumulation of its body reserves. Therefore, the efficiency of Pelibuey ewes should be evaluated considering the productivity (litter weight) associated with the intrapartum period.

CONCLUSIONS

Overall, ewe BW at lambing, position/insertion of the teats into the udder and litter type (sex and prolificacy) are factors controlling the offspring pre-weaning growth, as well as the ewe productivity and efficiency at lambing and weaning in Pelibuey ewes reared in tropical production systems. Given that farmers want greater productive efficiency at weaning, heavy Pelibuey ewes with non-misshapen udders and double litters could be of optimal maternal characteristics.

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Statement of animal rights. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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AP AGRO PRODUCTIVIDAD

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Laelia anceps Lindl. (Orchidaceae) adaptation on phorophytes within an anthropized landscape

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ABSTRACT

Objective: To determine the best phorophyte species for the adaptation of the *Laelia anceps* Lindl. orchid (Orchidaceae) in an anthropized landscape.

Design/Methodology/Approach: A completely randomized experimental design with four treatments (phorophytes) was used: *Swietenia macrophylla* King., *Fraxinus* sp., *Persea schiedeana* Nees., and *Tecoma stans*. (L.) Juss. ex Kunth), with five repetitions per phorophyte and with three *L. anceps* orchid plants in each phorophyte. The following variables were measured: day to emergence, root length and thickness, and survival at 28 weeks after tying them in phorophytes.

Results: Root emergence of *L. anceps* took place in less time in *S. macrophylla* and in *Fraxinus* sp. at 52 and 54 days, respectively. The longest root length (19.11 cm) and number of roots (32.45) were observed in *S. macrophylla*; however, the root thickness was greater in *Fraxinus* sp. (0.28 cm). After 28 weeks of establishing the *L. anceps* plants, 100% survival was obtained in the phorophytes *S. macrophylla* and *Fraxinus* sp., 77% in *P. schiedeana*, and 33% in *T. stans*.

Study Limitations/Implications: The amount of light received by *L. anceps* in each phorophyte was not measured.

Findings/Conclusions: The best phorophytes observed for the establishment of *L. anceps* were *S. macrophylla* and *Fraxinus* sp., with the best development and strength of the roots and 100% survival at 28 weeks.

Keywords: native trees, epiphyte, host, native orchid, orchid survival.

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INTRODUCTION

Orchids have been part of the national culture in Mexico since pre-Hispanic times. Within this family, the species *Laelia anceps* Lindl. has stood out for its beauty, wide use and distribution, which makes it one of the most appreciated ornamental species (Halbinger and Soto, 1997; Baltazar-Bernal *et al*., 2020). The flowering season coincides with the Day of the Dead festivities and it is widely used in altars (Halbinger and Soto, 1997), so it is considered a sacred orchid (Hágsater *et al*., 2015). In Veracruz it is known as *monjitas* or *calaveritas*.

Like most orchids, *L. anceps* has been severely affected by illegal harvesting and commercialization, but also by habitat loss and fragmentation (Mondragón *et al.*, 2015; Solano-Gómez *et al.*, 2007). Specifically, landscape fragmentation impedes the movement of orchid propagules by decreasing the phorophytes or trees that host them, in addition to relative humidity (Montibeller-Silva *et al.*, 2020). Therefore, the conservation of forests that harbor the greatest diversity of phorophytes with orchid communities is very important (Morales-Linares *et al.*, 2019). Because of the above, and given that orchids are very frequent common goods in urban landscapes, it is vital to understand the biophysical factors that influence their establishment and the conservation of these public spaces (Batty *et al.*, 2002). Among these biotic factors are the variations in microclimate necessary for the development of orchids and the organisms with which the orchids and their phorophytes interact, such as fungi, lichens and pollinators, which are strongly altered by anthropogenic activities (Besi *et al.*, 2019). In this sense, the reintroduction of orchids and other plants in ecosystem restoration programs is very important if a habitat similar to that found before the landscape was disturbed is to be recreated (Phillips *et al.*, 2020). It is also vital to include local communities or villages in forest restoration and conservation programs, particularly the phorophytes and orchids that inhabit them (Trimanto, 2020), in order for the programs to be more successful. These conservation efforts are important because landscapes with high plant cover are inhabited by a greater number of epiphytic species (Leighton *et al.*, 2016).

In the case of the orchidaceous flora in Veracruz, it constitutes 90% of the species with epiphytic growth (CONABIO, 2011), and the fundamental partners for this type of orchids are the phorophytes (Menchaca and Rendon, 2016). Phorophytes are mostly arboreal species that offer anchorage, support and provide characteristics (bark type, foliage density, crown shape) that help orchid development (Benzing, 2008; Granados *et al.*, 2003).

The best way to evaluate the quality of a phorophyte is the survival of an orchid established in it, as has been done in some studies. For example, regarding the *in situ* survival of the genus *Laelia*, Luyando-Moreno *et al.* (2011) evaluated the *in situ* survival of *L. autumnalis* in a pine-oak forest, specifically identifying *Arbutus xalapensis* as a good phorophyte for the epiphytic orchid *L. autumnalis*. On the other hand, Morales (2019) evaluated the survival of *in vitro* cultivated seedlings of *L. anceps* established in a tropical oak forest, for which he recommended placing the seedlings 5-6 m above the ground, in the oak trees. Apparently, no orchid survival studies have been carried out. Thus, taking into consideration the above, this study aimed to determine the best phorophyte species for the adaptation of *L. anceps* in an anthropized landscape, using native trees.

MATERIALS AND METHODS

Study area

The study was conducted in the gardens of Colegio de Postgraduados Campus Córdoba (CPCC), located in Amatlán de los Reyes, Veracruz at 18° 51' 21" N, 96° 51' 35" W, and an altitude of 627 masl. The climate is semi-warm and humid, with abundant rainfall in summer; the average annual temperature range is between 20 and 24 °C, and annual rainfall is 2150 mm (INAFED, 2021). The vegetation of the study area consists of tree species of mesophilic forest and lowland tropical rainforest, with native species such as *Annona* sp. (pawpaw), *Persea schiedeana* Nees. (Creole avocado), *Tecoma stans*. (L.) Juss. ex Kunth) (yellow elder) and *Fraxinus* sp. (ash), and introduced species such as *Azadiractha indica* A. Juss. (neem), *Mangifera indica* (mango), and *Citrus* × *lemon* (lemon) (Baltazar-Bernal *et al*., 2020), which are some natural or potential phorophytes of different types of epiphytic plants, such as orchids.

Plant material

L. anceps is a medium-sized orchid native to Mexico, producing 80 cm long flower stalks with an inflorescence composed of two to five flowers (Halbinger and Soto, 1997; Figure 1). It flowers from mid-October to early December. Because of its beauty, the variety of its colors and the size of its flowers, it is an orchid that is traded in traditional markets in the Campus Córdoba region (Baltazar-Bernal *et al*., 2020).

The study period was from November 2020 to June 2021. Four adult *L. anceps* plants showing damage from water stress, fungi, and sunburn were collected from areas adjacent to the campus. The plants were divided by cutting a section of the rhizome with four to six pseudobulbs, which were carefully washed with drinking water and then immersed in a water solution with Captan® 500 fungicide (1 g L^{-1}) for 10 min (Figure 2).

The average characteristics of the plants were: pseudobulbs 2.43 cm thick and 6.85 cm long, generally with three leaves 4.7 cm wide and 19.7 cm long, on average.

Establishment of the experiment

The prepared *L. anceps* plants were attached to the phorophyte trunk with plastic string at a height of 1.2 to 2.0 m in each of the four different phorophytes (Figure 3). The height of the phorophytes ranged from 5.6 to 10.9 m, the diameter at chest height (DCH) ranged from 25.3 to 57 cm (Table 1), and the different shades and textures of the bark (Figure 4). Moisture was maintained with manual water jet irrigation, twice a week.

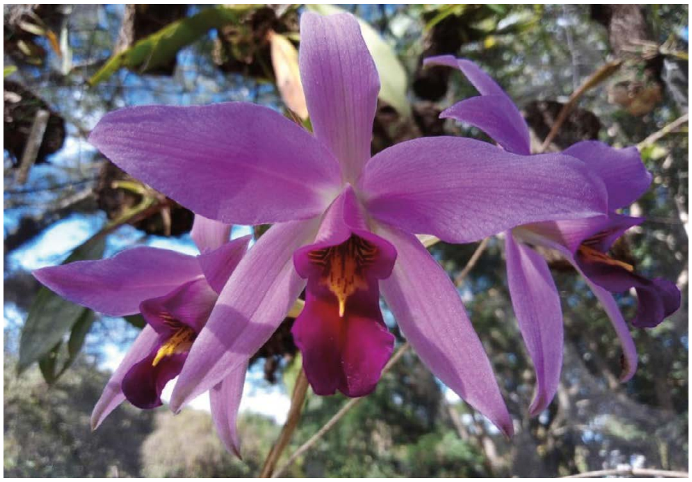

Figure 1. Inflorescence of *Laelia anceps* Lindl.

Figure 2. *Laelia anceps* Lindl. plant prepared for attachment to a phorophyte.

Figure 3. Establishment of *Laelia anceps* Lindl.

Table 1. Phorophyte characteristics selected for the study.

Scientific name (Common name)	**Height (m)**	**DAH (cm)**	**Bark type**	**Bark color**
Persea schiedeana Nees. (aguacate criollo)	6.1	54.1	Widely ribbed	Dark brown
Fraxinus sp. (fresno)	5.6	25.3	Fissured	Greyish brown
Swietenia macrophylla King. (caoba)	10.9	57.0	Slightly fissured	Greyish brown
Tecoma stans (L.) Juss. ex Kunth. (lluvia de oro)	7.2	54.0	Scaly and widely fissured	Dark brown

DCH=Diameter at chest height.

The average temperature during the study period was 21.7 °C. The average relative humidity was 73.1% (Figure 5).

Figure 4. Bark of the phorophytes used. A) *Persea schiedeana* Nees., B) *Fraxinus* sp., C) *Swietenia macrophylla* King., and D) *Tecoma stans* (L.) Juss. ex Kunth.

Experimental design and data analysis

The experiment was established in a completely randomized experimental design with four treatments (phorophytes): *Swietenia macrophylla* King, *Fraxinus* sp., *Persea schiedeana* Nees., and *Tecoma stans*. (L.) Juss. ex Kunth, with five replicates per phorophyte and three plants of the orchid *L. anceps* in each phorophyte. For each of the three specimens of *L. anceps*, the following variables were recorded: root emergence (number of days), root length (cm), root thickness (cm) and plant survival (percentage). These variables were measured 28 weeks after establishment of the orchids in the phorophytes. The data obtained were processed in the IBM SPSS Statistics software (version 21). An analysis of variance (ANOVA) was performed, followed by Tukey's test ($p \leq 0.05$), in order to know significant differences between treatments.

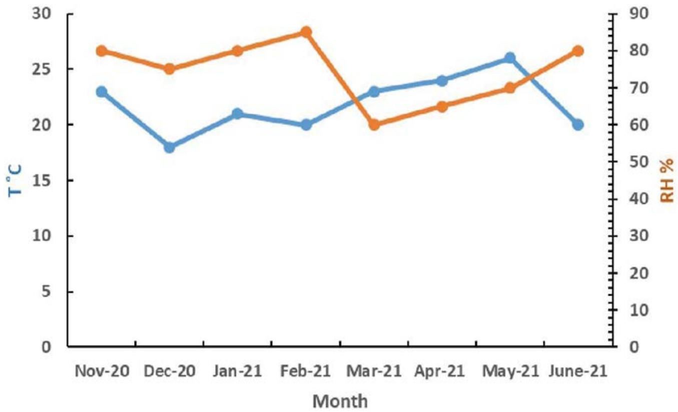

Figure 5. Temperature and relative humidity during the study period.

RESULTS AND DISCUSSION

After 28 weeks of establishing specimens of *L. anceps*, significant differences were observed in the different phorophytes evaluated. In root emergence, an early response was obtained in *S. macrophylla* and *Fraxinus* sp. trees, with 52 and 54 days, respectively, and a similar response was observed in root length of both *S. macrophylla* and *Fraxinus* sp., with 19.11 and 18.22 cm, respectively. The behavior of *L. anceps* on hosts *S. macrophylla* and *Fraxinus* sp. with fissured and slightly fissured bark, respectively, favored early emergence of new roots and longer root length. These observations are agreed with the research by Hernández-Pérez *et al.* (2018) in which a higher richness and abundance of orchids was found in 23 species of fissured-bark trees and a lower richness in 12 species of smooth-bark trees. Similarly, Alzate-Q *et al.* (2019) found that the phorophyte *Clethra macrophylla* presented a higher richness of epiphytic species (41%) related to its multiple branches and rough bark. In contrast, studies by Zotarelli *et al.* (2018) showed that the bark of the phorophyte does not influence the number of orchids it can host. This would seem to reject the suggestion that the type of bark of both phorophytes does not positively influence root emergence in the specific case of *L. anceps*, since they have fissured bark, which may be ideal for root development.

The root thickness of *L. anceps* was very similar to that of *Fraxinus* sp. (0.28 cm), *S. macrophylla* (0.25 cm) and *P. schiedeana* (0.22 cm), and clearly superior to *T. stans* (0.06 cm). For the number of roots, similar results were also obtained for the same three species, which are also superior to *T. stans* (Table 2).

The poor root development of *L. anceps* in the *T. stans* phorophyte may be linked to the type of scaly, light and widely fissured bark that hosts few epiphytic plants, compared to the other phorophytes. This seems to agree with the findings by Yam *et al.* (2014), who found a lower presence of orchids and other epiphytic plants in phorophytes with soft (light) bark, within an urbanized landscape.

Regarding the survival of *L. anceps* plants in the phorophytes, in *S. macrophylla* and *Fraxinus* sp. there was 100% survival, in *P. schiedeana* there was 77% survival, and in *T. stans* there was only 33% survival (Table 2).

A possible reason for the differences in survival may be due to the morphological characteristics of the bark of *T. stans*, which do not facilitate orchid establishment, because

Table 2. *Laelia anceps* Lindl. development in four phorophytes in five variables.

Phorophyte	Root emergence (days)	Root length (cm)	Root thickness (cm)	Root number	Survival (%)
Persea schiedeana Nees. Aguacate criollo	78.38±7.80 b*	8.89±2.01 b	0.22±0.04 a	17.67±3.60 b	77±5.92 b
Fraxinus sp. Fresno	54.89±7.50 a	18.22±0.81 a	0.28±0.01 a	26.71±2.75 ab	100±0.00 ab
Swietenia macrophylla King. Caoba	52.56±1.60 a	19.11±2.62 a	0.25±0.01 a	32.45±2.57 a	100±0.00 a
Tecoma stans (L.) Juss. Ex Kunth Lluvia de oro	99.78±0.22 c	1.11±0.77 c	0.06±0.03 b	0.89±0.88 c	33±6.73 c

The mean ± standard error. * The means of the columns followed by different letters are statistically different (Tukey, $p \leq 0.05$).

it retains less moisture than the other species, given that all treatments received the same amount of irrigation. Segovia-Rivas *et al.* (2018) pointed out that humidity plays a decisive role in the acclimatization of orchids in phorophytes, so it is recommended to apply constant irrigation, when environmental conditions are very dry.

The data suggest that phorophyte type and constant irrigation can ensure the survival of *Laelia anceps* in disturbed rural landscapes. Coupled with the above, Einzmann and Zotz (2017) conclude that orchids can overcome connectivity barriers in the landscape. The authors also pointed out that the size and diversity of the landscape fragment are factors that define the presence of the microclimatic conditions necessary for their development. For Izzudin *et al.* (2018), the reintroduction of native orchids in anthropized landscapes is a viable method to ensure the continuity of species. In addition, they noted that efforts should be made to make appropriate management of these fragments to convert them into refuges for native epiphytic orchids which is in agreement with Nurfadilah (2015).

CONCLUSIONS

The data from this experiment suggest that adaptation of *L. anceps* plants in an urbanized environment is possible, if suitable phorophyte species are determined to allow good root development. In this sense, the best phorophytes for the establishment of *L. anceps* were *S. macrophylla* (mahogany) and *Fraxinus* sp. (ash) with 100% survival, given their greater root development.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Characterization of black mangrove (*Avicennia germinans*) and red mangrove (*Rhizophora mangle*) ecosystems in Paraíso, Tabasco, Mexico

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ABSTRACT

Objective: To characterize black and red mangrove ecosystems in the coastal zone of Paraíso, Tabasco, Mexico.

Design/Methodology/Approach: Four plots were randomly established in each mangrove ecosystem. Inside each plot, trees were counted per transect, and measured for basal area, diameter at breast height, plant cover and height. Seven diametric classes were estimated to calculate the relative species richness and the dynamics of emergence/disappearance in the ecosystem. Soil samples were surveyed at two depths and during different seasons (dry and rainy). The samples were tested for organic matter (OM), nitrogen content (N), phosphorus (Olsen P), pH, exchangeable bases and humic and fulvic acids; with the objective to characterize nutrient flux dynamics between seasons and compare the two mangrove ecosystems.

Results: Red mangrove ecosystems contain higher OM during rainy season. Black mangrove ecosystems showed low contents of OM and exchangeable bases during dry season. The species with more individuals m^{-2} is red mangrove, and black mangrove presents individuals with greater basal area and diameters.

Study Limitations/Implications: Regional characterization of mangrove ecosystems provides information about nutrient flux, which is necessary for the adequate proposal of protection and conservation programs of these wetlands.

Findings/Conclusions: The dominant mangrove species affects nutrient flux in soils associated to the ecosystem, suggesting they have specific functions in the ecosystem dynamics.

Keywords: Wetlands; coastal ecosystems; organic matter; nutrients flux; forestry richness.

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INTRODUCTION

Mangroves are coastal wetland ecosystems that occupy a privileged place because of their natural wealth and the environmental services they provide, in addition to representing an important ecological role that has been recognized internationally. They are valuable natural ecosystems that face severe alterations, such as contamination from the discharge of

Image by Suzanne D. Williams at Pixabay

residual waters and the overexploitation of products derived from these, which affect their structure, functioning and their very existence (Domínguez-Domínguez *et al.*, 2011). In Mexico there are five species of mangrove: *Rhizophora mangle* L. (red mangrove), *Avicennia germinans* L. (black mangrove or dark mangrove), *Laguncularia recemosa* L. (white mangrove), *Conocarpus erectus* L. (botoncillo mangrove) and *Rhizophora harrisonnii* (caballero mangrove) (Corella-Justavino *et al.*, 2004; Domínguez-Domínguez *et al.*, 2011). All of them are found in the category of special protection NOM-ECOL-059/2001 (SEMARNAT, 2002). The state of Tabasco has 189 km of coast, where coastal lagoon systems are located, among which there are 40 km^2 of mangrove forests (Pérez-Sánchez *et al.*, 2002; Valderrama-Landeros *et al.*, 2017).

The mangroves in the municipality of Paraíso, Tabasco, have special importance for rural communities, not only as traditional forest resource; currently their importance is centered in various ecosystem services that they provide to improve the quality of life of nearby populations (Sánchez *et al.*, 2016). Understanding the characteristics of the mangrove ecosystems in the coast of Paraíso, Tabasco, could be the basis for the characterization and identification of the ecosystem benefits that they can be providing for human settlements, as well as the implementation of strategies that ensure their sustainable exploitation and their conservation. The objective of this study was to analyze and compare two mature mangrove ecosystems in Paraíso, Tabasco, one of red mangrove (*R. mangle*) and another of black mangrove (*A. germinans*) in their plant structure and nutritional characterization of the soil.

MATERIALS AND METHODS

Study area. The municipality of Paraíso is located in the region of the Grijalva River, in the sub-region known as Chontalpa in the state of Tabasco, at an average altitude of 10 meters above sea level. It has a territorial surface of 377.55 square kilometers (Domínguez-Domínguez *et al.*, 2011). The climate is warm-humid with abundant rainfall during the summer, with the months of May to August being the warmest, with temperatures that exceed 34 degrees centigrade (Palma-López *et al.*, 2007). The study was performed in the locality called Nicolás Bravo 4th section, from this municipality, where the Tilapa Lagoon is located. The coordinates are 18° 20' 33" latitude North and 93° 07' 39" latitude West.

Experimental design and field sampling. Two plots of size 40×20 were established in *R. mangle* and of 30×30 m^2 in *A. germinans*, due to the location of each species. Plant sampling was done on September 3, 2019, with the transect method (Mostacedo & Fredericksen, 2000); for this purpose, four transects of 15 m length by 10 m width in each mangrove plot were delimited with raffia palm thread. In each sampling unit an inventory of all the trees was conducted; of all the trees with basal diameter ≥2.5 cm the following data were recorded: diameter at breast height (DBH), with the help of a diameter tape; height and percentage of the coverage with a Haga gun. In each transect the number of trees of smaller size was also counted, while in *R. mangle* they were measured 30 cm above the last aerial root (Corella-Justavino *et al.*, 2004). The field information was systematized in the Excel 2016 software, where a database was generated, which was used to estimate the density of vegetation per hectare of each sampling unit. According to Velázquez-Pérez *et al.* (2019), seven diameter classes were established (2.5-4.9, 5-7.4, 7.5-9.9, 10-12.4, 12.5-14.9, 15-19.9 and >20 cm), in which the individuals sampled were placed, which allowed estimating the relative abundance of the species and the dynamics of emergence and disappearance of them in the ecosystem. The basal area (*BA*) and the trunk volume (*V*) were calculated through the following formulas (Mostacedo & Fredericksen, 2000):

$$BA = p(D2/4)$$

where: $p=3.141592$, and D refers to the diameter at breast height (DBH).

$$V = BA*h$$

where h refers to the total height of the trunk.

Soil sampling and analysis. The types of soils in each ecosystem were identified, based on the Global Soil Resource Referential Base (IUSS, 2015) and taking as reference the information from Palma-López *et al.* (2007). The soil samples were taken on days June 6, 2019 (dry season), and September 24, 2019 (rainy season). Samples were taken with Dutch type drill at depths of 0-30 cm and 30-50 cm, each one of the two samples was made up of 15 sub-samples taken randomly in zig-zag for the two ecosystems. The chemical analysis of the soils performed were CIC (extraction with ammonium acetate), MOS (moist combustion Walkley and Black), total N (Semi-micro Kjeldahl, modified to include nitrites), P (Olsen), PH ($CaCl_2$, 1:2), exchangeable bases: (Na, K, Ca, Mg) (extraction with ammonium acetate 1.0 N to pH 7.0) according to the methodology of NOM-021-RECNAT-2000 (SEMARNAT, 2002); and lastly, humic and fulvic acids (Kononova-Belchikova method).

RESULTS AND DISCUSSION

Soil characterization. The soils were classified as: Protosodic Stagnic Gleyic Solonchak (Clayic, Humic) for the ecosystem of black mangrove (*Avicennia germinans*) and Fluvic Sodic Stagnic Gleyic Solonchak (Clayic, Histic, Humic, Hypersalic) for the ecosystem of red mangrove (*Rhizophora mangle*) (IUSS, 2015). In both cases they are salinized soils where the processes of gleization and blockage of superficial water are dominant. In the ecosystem of *R. mangle*, the presence of salinity and sodicity is stronger, which coincides with what was reported by Palma-López *et al.* (2007) and Domínguez-Domínguez *et al.* (2011).

The two mangrove communities studied were made up only by the species that receive their name, and only in red mangrove was a single immature individual observed of the climbing plant *Rhabdadenia biflora* (Jacq.) Müll. Arg. The density of *A. germinans* was 1,633 individuals per hectare, higher than in *R. mangle*, whose density reached only 633. Domínguez-Domínguez *et al.* (2011) reported in the state of Tabasco that black mangrove was scarcer in number of individuals; in the characterization of the mangrove in two localities near the studied area, the same authors indicated a density lower than 308 trees per hectare for black mangrove and 279 for red mangrove. In contrast, Corella-Justavino *et* *al*. (2004) reported 1,109 and 774 trees of red and black mangrove per hectare, respectively, in Centla swamps. In Chiapas mangroves, the average density was 2,915 individuals ha^{-1}, which were made up of young-mature *A. germinans* trees, located in salinized sites and with strong wood extraction of *L. racemosa* (Velázquez-Pérez *et al*., 2019).

Figure 1 shows the frequency of the diametric classes in the two ecosystems of study; in the case of *A. germinans* the distribution was characterized by presenting a higher concentration of individuals in the first category (2.5-4.9), with a tendency to decrease towards the last classes. Instead, *R. mangle* showed a more homogeneous distribution between the different classes, being slightly higher in the one of 7.5-9.9 cm, and the average DBH for those communities were 7.33 and 13.21 cm, respectively. In this sense, Corella-Justavino *et al*. (2004) found the highest density in the first category in both species. In contrast, in the analysis of the mangrove structure in Mecoacán Lagoon, Domínguez-Domínguez *et al*. (2011) mentioned that for Ejido Libertad 1st Section, red mangrove reflects an adequate incorporation of young individuals and a progressive decrease of these as they grow in age and diameter, while for black mangrove it presents an alarming scarcity of young individuals and trees that can reach 50 cm of diameter, which contrasts with the results of this study. However, the same study mentions that red mangrove has a very balanced diametric distribution in Ejido Campo Mecoacán, while black mangrove had an adequate young population for the size of its population, being evident that many black mangroves of older age have been respected.

Regarding the height of trees, Figure 2 shows their distribution in both communities studied, where a taller size is appreciated in red mangrove, whose average was 17.6 m, while black mangrove barely reached 13.88 m. Height is an indicator of the development of the structure of a stand, since a greater height is related with a higher DBH. Contrary to these data, in Centla Swamps the species that had the highest average height was *A. germinans*, followed by *L. racemosa*, the one of smallest size was *R. mangle* (Corella-Justavino *et al*., 2004). Likewise, Domínguez-Domínguez *et al*. (2011) reported ranges of 12 to 19 m for black mangrove in Tabasco, with values of 19 and 11 m for Ejidos Libertad 1st Section and Campo Mecoacán, sites close to the study area, respectively; and ranges of 9 to 16 m for red mangrove, and values of 15 and 16 m for the same *ejidos*. Sánchez *et al*.

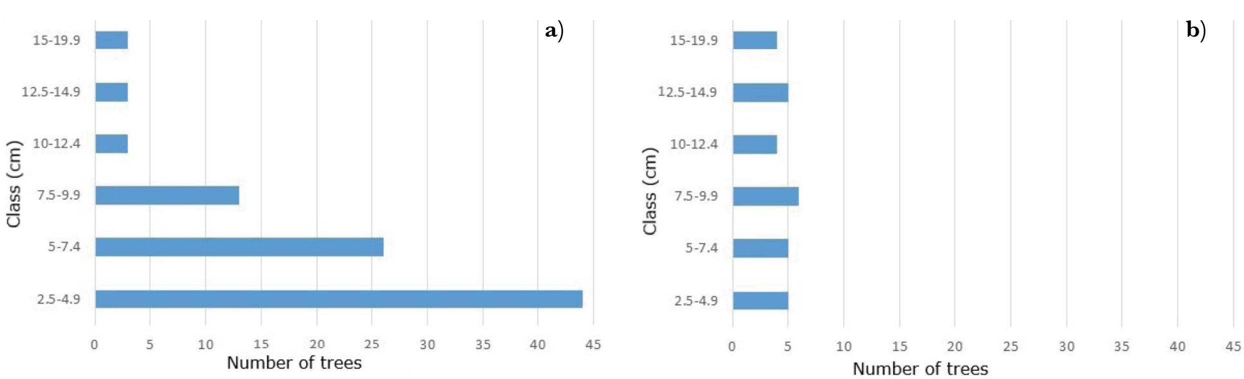

Figure 1. Distribution of trees by diameter class in two mature mangrove ecosystems: a) black mangrove, and b) red mangrove, both in Paraíso, Tabasco, Mexico.

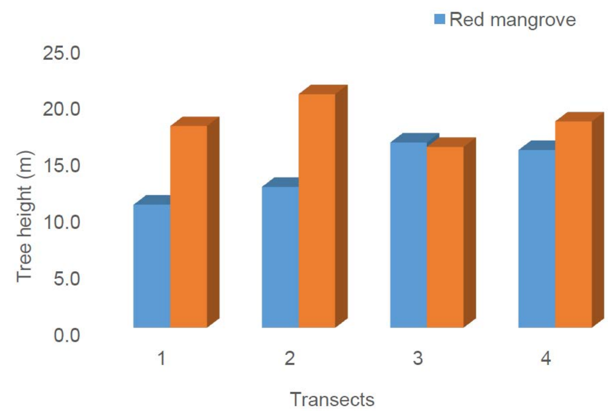

Figure 2. Tree height in sampled mangrove communities (two transects per season).

(2016) consider that the height in itself is not a limitation for their exploitation; however, it suggests that trees with mean heights lower than 10 meters are still not profitable for their exploitation, which is why respecting them and caring for their growth until they reach an adequate height is the suggestion.

Chemical properties of the soil associated to mangrove ecosystems. The average values of the chemical properties of soils at 0-50 cm depth in the ecosystems studied and in the two seasons (dry and rainy) are shown in Table 1. The pH showed values classified as neutral for black mangrove, and strong to moderately acid in red mangrove (SEMARNAT, 2002). The pH tends to be acid in tropical soils, and even more in those of mangrove (Sánchez-Arias *et al.*, 2010); the anaerobic metabolism in the natural mangrove ecosystem occupied with flooded soils rich in sulfates, where its reduction is the dominant process, making the soil generally acid (Senior *et al.*, 1982; Palma-López *et al.*, 2007; Adame *et al.*, 2018).

The percentages of organic matter (OM) found were middle in black mangrove and high in red mangrove (SEMARNAT, 2002); this behavior seems to be due to the larger population and therefore the higher deposition of organic residues, with an increase of OM observed in both ecosystems during the rainy season. The seasonal variations of temperature, hours of sunshine, and level of flooding influence the fall of different parts of the plants, primarily leaves, and therefore in the increase of OM (Bernini *et al.*, 2010; Alongi *et al.*, 2014).

Table 1. Soil chemical properties of red mangrove (*Rhizophora mangle*) and black mangrove (*Avicennia germinans*) ecosystems spanning two seasons a year.

Season	Ecosystem	pH (H_2O) Rel. 1:2	OM	N	P	K	Ca	Mg	CEC	HA	FA
			%		mg kg^{-1}	cmol$_{(c)}$ kg^{-1}				%	
Dry	Black mangrove	6.5	2.4	0.10	5.7	0.2	12.5	3.2	27.1	0.9	2.3
	Red mangrove	4.9	4.6	0.17	8.2	0.3	14.9	5.3	32.3	1.3	2.57
Wet	Black mangrove	6.7	3.5	0.17	6.8	0.4	15.0	5.5	30.8	1.1	2.40
	Red mangrove	5.2	8.1	0.35	10.7	0.5	15.3	8.0	33.3	2.1	3.42

OM=Organic matter; N=nitrogen (Kjeldahl); P=Phosphorus (Olsen); K=Potassium; Ca=Calcium; Mg=Magnesium; CEC=Cation Exchange capacity; AF=Fulvic acids; HA=Humic acids.

The N contents in black mangrove were middle and in red mangrove high (Table 1), being higher in the rainy season, and these values have correspondence with the levels of OM. Red mangrove (*R. mangle*) remains flooded longer during the rainy season and has higher OM deposition (Sánchez-Junco *et al.*, 2011; Fernandes *et al.*, 2012, Romero *et al.*, 2012).

Phosphorus presented middle values in the two mangrove ecosystems and in the two seasons, dry and rainy (Table 1); however, the highest average values were found in red mangrove (9.5 mg kg^{-1}) (SEMARNAT, 2002). Phosphorus is an element that in tree agroecosystems tends to present middle values, and this is related to the property that trees have to extract this element from great depths in the soil (Middleton & Mckee, 2001; Sánchez *et al.*, 2016).

The exchangeable bases presented variations that seem to be influenced by the season, showing an important increase in K (40% in the two ecosystems) and Mg in its content for the rainy season (58% black mangrove and 66% red mangrove), and also in Ca for that same period, showing a higher concentration although less pronounced. The contents of K in the two ecosystems during the dry season were very low to low, while during the rainy season they were middle to high. And in seasons they were very low to low, contrary case to Mg and Ca, which had high contents (SEMARNAT, 2002).

The cationic exchange capacity (Table 1) in the soils studied was high (SEMARNAT, 2002). The presence of the mangrove species, the type of soil, the constant contributions of residues from deposition, as well as those related to plant coverage, favor soil fertility (Dinesh *et al.*, 2004). In this regard, Bautista-Cruz *et al.* (2004) mention that the cationic exchange capacity increases when there is high presence of organic matter and the CEC presents higher values, which vary from 25 to 40 Cmol kg^{-1}.

The contents of humic and fulvic acids (Table 1) in the agroecosystems studied were high. These are part of the complex organic matter system of the soil (SOM), they are product of the partial decomposition that results from the microbiological and chemical transformations, and present a high buffering capacity (Singh *et al.*, 2005; Kida & Fujitake, 2020).

CONCLUSION

The soil from the two mangrove ecosystems corresponded to saline soils dominated by the gleization processes, stagnation, and in both sites it was classified as Solonchaks. In diametric aspects, the species of *R. mangle* presented values with higher DBH, height and plant coverage. The analyses generated indicate that the vegetation and the type of species influence the nutritional results of the soil, since each fulfills a specific function that provides good development of the mangrove species, and they also influence the seasons of the year in each ecosystem, and the greatest development of *R. mangle* when comparing its dasometry with *A. germinans.*

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AP AGRO PRODUCTIVIDAD

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Impact on the soil and the infiltration as a consequence of oil palm cultivation (*Elaeis guineensis* Jacq.) in Tabasco

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ABSTRACT

Objective: To evaluate the possible negative impacts on soil fertility, as a result of the soil use change from the grassland to oil palm.

Design/methodology/approach: The following variables were evaluated: soil organic matter (SOM), bulk density (BD), mechanical penetration resistance (MPR), root system distribution, and infiltration in 5-, 11- and 25-year-old oil palm plantations, as well as in an adjoining grassland —whose land use had not changed to oil palm cultivation.

Results: During the first years of cultivation, the substitution of the grassland for oil palm caused SOM losses, increased BD and MPR, and reduced infiltration levels. After 11 years, these effects became stable and were reversed. Therefore, in mature plantations of >25 years, the soil and infiltration conditions improved, even surpassing the grassland. These changes occur at a depth of 40 cm and are attributable to the SOM provided by the root system; consequently, root distribution does not block infiltration, becoming a beneficial factor, particularly in mature plantations.

Study limitations/implications: The research must be replicated under other soil conditions, in order to observe the fertility behavior.

Findings/conclusions: After the grassland is replaced by oil palm, soil fertility deteriorates during the first years; fertility becomes stable and recovers after 11 years. Meanwhile, the effect reverses and surpasses grassland fertility levels after 25 years.

Keywords: Soil use change, Environmental impact, Soil conservation, Water infiltration.

INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a highly productive oleaginous plant. During the last decades, the area in which this crop is cultivated has rapidly increased worldwide. These plantations have spread in southern-southeastern Mexico, where the edaphoclimatic conditions favor its development and this trend will likely continue (Hernández-Rojas *et al.*, 2018). In Tabasco, the cultivated area grew during the last decade, raising questions about

Image by Bishnu Sarangi at Pixabay

the real environmental impact of this crop. Therefore, international standards have been issued aimed at achieving a more harmonious relationship between oil palm cultivation and the environment. These standards include the Roundtable on Sustainable Palm Oil (RSPO) and, in Mexico, the NMX-F-817-SCFI-2020 ACEITE standard, which was issued in 2020. This Mexican standard establishes the requirements and specifications about the sustainable oil palm value chain. Both standards attempt to establish the principles and criteria of a sustainable oil palm cultivation. However, these are general guidelines that must be adjusted to the specific conditions of each productive zone. Therefore, in order to find out the impact of oil palm cultivation on the environment, we must understand the interactions between the biotic and abiotic factors of the ecosystems. Consequently, climate, soil, plant, and geo-hydrological factors variables must be included in the researches. Determining the impacts of oil palm cultivations on the water and edaphic resources of the productive areas in Tabasco require comprehensive studies, in which interdisciplinary efforts must come together. In this context, researches aimed at evaluating possible negative impacts of oil palm crops on the soil conservation are already underway. Therefore, this article includes some advances of these researches and a bibliographical analysis which answers some questions about the impact of oil palm crops. Consequently, this research opens new paths for the development of a future sustainable oil palm cultivation in the State of Tabasco.

MATERIALS AND METHODS

The study area is located in the third section of the Chipilinar rural settlement, in the Jalapa municipality, Tabasco, at 17° 46’ 52.8” N and 92° 46’ 05.4” W, with an average altitude of 20 masl. The annual rainfall average is 3,783 mm, with a maximum monthly average of 728 mm (September) and a minimum monthly average of 81 mm (April) (CONAGUA, 2020). Several soils variation can be found, including sandstone lutite in the surface and polymic conglomerate lutite in the deep (Zavala-Cruz *et al.*, 2016). Based on the WRB nomenclature description of profiles and the physical and chemical analysis, the soil is classified as Chromic Lixisol (Clayic, Densic, Differentic, Humic, Profondic) (2014).

Experiment description and study variables

Three contiguous oil palm plantations of different ages were selected, as well as an adjoining grassland. The following treatments were evaluated: a 5-year-old young plantation (Treatment 1); an 11-year-old full production plantation (Treatment 2); a 25-year-old plantation (Treatment 3); and an adjoining grassland (Treatment 4). Treatment 4 maintained the original grassland use and was considered as control.

Samples were collected following a zigzag route, until the total surface was covered. Each area provided two series of 20 samples. In order to determine SOM, the first set of samples was collected at 4 different depths (0-40, 40-80, 80-120, and >120 cm) (Walkley and Black, 1932). In order to determine BD, the second set of samples was obtained using a soil-push probe with a known volume cylinder (Blake and Hartage, 1986). At the same time, the mechanical penetration resistance (MPR) was tested in the same sampling areas, using a cone penetrometer (Dexter *et al.*, 2007).

An adaptation of the Hagg (1997) method was used to describe the root system distribution: three plants were selected per each treatment. Three trial pits were dug next to each palm. Cartesian grids were marked out in the walls of the trial pits. Samples were taken at four depths (0-40, 40-80, 80-120, and >120 cm) in the Y vertical axis. Meanwhile samples were taken at six different distances from the stipe base (stem) in the X horizontal axis (0, 50, 100, 150, 200, and 250 cm). The soil samples from each XY grid were dried on a forced air oven at 105 °C. Subsequently, the samples were weighted and crushed and the roots were separated using a double sifter: the first (dry), with a number 18 sift (1 mmØ); the second (wet), with a number 25 sift (710 μmØ). The roots obtained from the second sifting were dried and added to the roots obtained from the first sifting. The root percentage of each sample was obtained using the relation between the weight of the dry root mass and the initial total mass weight of each soil sample, multiplied by 100. In order to determine the infiltration rate, three repetitions were carried out in each study area, using the doble ring infiltrometer method described by Kostiakov-Lewis (Delgadillo *et al.*, 2016).

The information obtained was processed using an analysis of variance (ANOVA), a correlation analysis, and Tukey tests. The SAS statistical software version 6.12 for Windows was used.

RESULTS AND DISCUSSION

Soil changes that can be attributed to oil palm

Organic matter (SOM) —the world's highest carbon reservoirs— is one of the major indicators for the evaluation of the impact that crops have on the soil (Gallardo, 2017). Table 1 shows the impact that crops have on some edaphic quality indicators.

Table 1 shows that the greatest changes in SOM and bulk density (BD) dynamics take place in the upper layers, particularly up to a depth of 40 cm. During the first cultivation years, the SOM percentage falls below the percentage of its initial condition —when the currently oil palm plantations were grasslands. This reduction in SOM contents is reversed

Table 1. Soil Organic Matter (SOM) content and bulk density (BD) levels in oil palm plantations of different ages respect to an adjacent grassland, according to the depth of the soil.

Treatments	Variables	Soil depth (cm)			
		0-40	40-80	80-120	>120
Oil Palm 5 years	SOM (%)	►1.65±0.20 b	0.74±0.24 b	0.45±0.17 b	0.33±0.08 a
Oil Palm 11 years		2.21±0.46 b	0.58±0.28 b	0.25±0.07 b	0.00±0.00 b
Oil Palm 25 years		4.68±1.18 a	1.11±0.06 a	0.92±0.29 a	0.32±0.10 a
Grassland		3.47±0.61 a	1.53±0.19 a	1.17±0.18 a	0.61±0.06 a
Oil Palm 5 years	BD (Mg m^{-3})	1.43±0.20 a	1.56±0.04 a	1.56± 0.10 a	1.57±0.06 a
Oil Palm 11 years		1.33±0.06 a	1.22±0.07 c	1.24±0.11 b	1.27±0.06 b
Oil Palm 25 years		1.04±0.16 b	1.40±0.11 b	1.42±0.18 a	1.54±0.07 a
Grassland		1.29±0.14 a	1.45±0.07 b	1.38±0.13 a	1.38±0.06 a

Equal letters mean they are statistically equal to a probability level of $p \leq 0.05$.
► Mean comparisons are made within each layer or depth.

in >25-year-old plantations, which even exceed the grassland's SOM contents. However, the said contents can only be found in the 0-40 cm layer; the effect is watered down at a greater depth, although this trend remains, with a less marked effect, at the deepest layers. In some soil use changes —for example, agroforestry systems deforested to set up grasslands—, SOM increases during the first years after the change; afterwards, the effect fades away. Salvador-Morales *et al.* (2017) point out that the low carbon-to-nitrogen ratio of the waste that becomes part of the soil is responsible for this situation: at a 12-15 ratio, waste decomposes at a fast pace, releasing nutrients and non-mineralized remainders. This remainder accumulates low SOM quantities which become part of the soil; however, waste with high carbon-to-nitrogen ratios (18-24) causes decomposition to take place at a slower pace and higher MOS content tends to accumulate. The said SOM accumulation entails several physical benefits, such as an improved soil structure, as a consequence of the increase of stable macroaggregates —which improve porosity and infiltration, reduce compaction (as bulk density (BD) decreases), improve rhizosphere conditions, and reduces hydric erosion (Sánchez-Hernández, 2017). The effect of this benefit is clearer in the topmost layer (Table 1), where the SOM concentration is higher; BD decreases in >25-years old plantations, reaching lower levels than the grassland. According to Sánchez-Hernández *et al.* (2017), the formation of aggregates can mainly be attributed to SOM; however, the most resistant or humidified SOM provides stability to the aggregates and consequently to the structure —as well as other properties that are linked to the said structure, such as BD and compaction. They also point out that a greater supply of organic waste in the soil modifies the size and stability of the aggregates, improves hydraulic conductivity (K), and diminishes penetration resistance (compaction), particularly in surface soil. However, they warn that these effects are not permanent; modifications take place as fresh organic waste runs out.

Figure 1 shows the compaction levels of the evaluated plantations, as well as of the adjoining grassland.

Therefore, up to a depth of 35 cm, 25-year-old plantations and the grassland keep the lowest compaction levels. At a >35 cm depth, only the grassland maintains low compaction

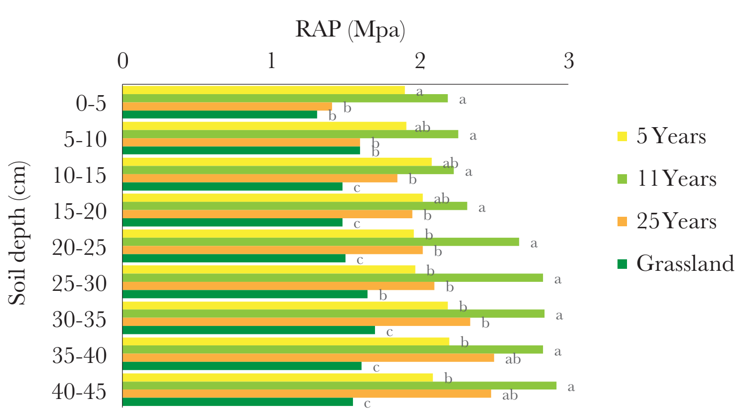

Figure 1. Soil compaction levels expressed as Resistance Against penetration (RAP) in Oil palm plantations and an adjacent grassland. Equal letters mean they are statistically equal to a probability level of p≤0.05. Mean comparisons are made within each layer or depth.

levels. However, compaction levels do not exceed 3 MPa —the critical value that prevents an appropriate root development— in any plantation, although compaction levels come close to those crucial levels, at a ≥35-cm depth.

Root system distribution

Table 2 compares the volume of roots with the volume of the sampled soil. Significative differences (Duncan $P<0.05$) were found, depending on the age of the plant.

Therefore, regardless of the age of the plant, its roots are distributed in a cone-shaped pattern. The volume of the root system is mostly distributed up to 40 cm deep and one meter around the trunk (Table 3).

These results match the findings of Ortiz and Fernández (2000) who reported that most of the volume of the root system —which is basically made of horizontally-growing radicles with anchoring functions— is found up to a depth of 50 cm. According to Arias (2020), several factors impact root growth and development. Regulating temperature —which can be achieved through plant coverage during the first years of life— favors the oil palm rhizosphere. On the contrary, rain-saturated soil can reduce the oxygen available for roots and can cause damage, as their susceptibility to pests and diseases increases (Vignola *et al.*, 2017). Soil porosity also affects roots. Low porosity has a negative impact on root growth; <50% total porosity can reduce root density up to 87% (Arias, 2020). Meanwhile, water deficit in the soil can diminish plant yield and growth, as a consequence of the stomatal closure that reduces the photosynthetic rate, interferes with carbon dioxide assimilation, and causes female inflorescence abortion (Vignola *et al.*, 2017).

Table 2.

Plantation age	Roots/soil (%)
5 years	0.09 b
11 years	0.21b
25 years	0.55 a

Equal letters mean they are statistically equal to a probability level of Duncan $p \leq 0.05$.

Table 3. Roots distribution of Oil palm (*Elaeis guineensis* Jacq.) to different Soil depth and stem distances.

Soil depth (cm)	Roots/Soil (%)	Distances (cm)	Roots/Soil (%)
0-40	0.7922 a	0	0.4450 a
40-80	0.2100 b	50	0.3642 a
80-120	0.0944 b	100	0.2725 a
>120	0.0561 b	150	0.2208 a
		200	0.2242 a
		250	0.2025 a

Equal letters mean they are statistically equal to a probability level of Duncan $p \leq 0.05$.

Infiltration rate

Several elements impact water flow in the soil and have repercussions on oil palm cultivation, including the calculation of the infiltration rate and the strip tillage which play a crucial role, as key components of the hydrological cycle (Luna *et al.*, 2020). There were no significative differences in basic infiltration rates (Table 4). However, according to the mean data, the age of the plant does impact the infiltration rate: a faster infiltration rate can be observed as the age of the oil palm crop increases. This can be caused by the increase of the root area and the OM of the oil palm crop (Table 1) which, in comparison with pasture, improve soil porosity and root content (Arias, 2020), consequently facilitating water flow. These data match other studies which point out that the infiltration rate is greater in cultivated soils than in bare soil, reducing the volume of water that is lost through evaporation and increasing the volume of water resources available for plants (Tapia *et al.*, 2020). However, some authors point out that soil uses associated with anthropic activities —such as agriculture, grazing, and forest management— can have a negative impact (Luna *et al.*, 2020). On the contrary, our infiltration rate data point out that palm has improved some properties.

Meanwhile, compared with the grassland used as control (Table 4), there are significative differences ($p<0.05$) with regard to strip tillage, which is clearly affected by the age of the plant.

Consequently, the plant's physiological process could require more water; the reduction of the humidity content in the soil enables water penetration. In this regard, Luna *et al.* (2020) point out that the crop's characteristics—including the ripeness degree, structure, and composition of the plant— and the edaphic variables —bulk density, organic layer thickness, and humidity— cause variations in the infiltration rate.

CONCLUSIONS

The land use change from grassland to oil palm cultivation has a negative impact on the soil during the first years in which that crop is grown: soil organic matter (SOM) is lost and BD and compaction increase, while infiltration rate also diminishes. Those effects become stable and revert to the original conditions 11 years later; particularly, >25-year-old plantations recover SOM and improve the abovementioned variables, sometimes even surpassing the grassland conditions. Most changes take place at a 0-40 cm depth and they can be attributed to the SOM content provided by the root system. Although the root system is superficial, it does not represent any kind of impediment to infiltration;

Table 4. Infiltration speed and water level accumulated in a period of 12 hours.

Treatments	Infiltration speed basic (cm hr^{-1})	Water level accumulated (cm)*
5 years	3.13 (±4.66) a**	23.72 (±6.97) ab
11 years	1.94 (±1.60) a	44.91 (±29.36) ab
25 years	5.61 (±3.20) a	75.83 (±35.48) c
Grassland	1.74 (±0.76) a	16.07 (±6.97) a

*For a time of twelve hours, ** ($p<0.05$).

on the contrary, it plays a beneficial role in mature plantations, improving the soil and consequently infiltration.

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AP AGRO PRODUCTIVIDAD

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Earthworm services: an agroecological perspective

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ABSTRACT

Objective: To review and discuss the invisible and silent role of the activity of earthworms, which are considered "agroecosystem engineers".

Design/methodology/approach: A database search of scientific articles published in Mexico and worldwide was performed.

Results: The literature reviewed shows that earthworms constitute the most abundant biomass in the vast majority of tropical agroecosystems. In addition, earthworms have long been shown to improve a) soil structure; b) interaction with symbiotic microorganisms; c) nutrient mineralization and availability; d) pollutant removal; e) regulation of foliar herbivory; and f) crop yields.

Limitations on study/implications: Most studies have been conducted in laboratories and greenhouses.

Findings/conclusions: Earthworms increase soil quality and agricultural productivity. Their protection, promotion, and management are suggested in order to manage, preserve, and restore soil health, as well as to ensure a sustainable crop production.

Keywords: Regenerative Agriculture, Environmental Services, Traditional Agriculture, Bioindicators, Bioremediation.

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INTRODUCTION

The presence of earthworms in crop fields is as old as agriculture. Plants have likely evolved along with earthworms (Blakemore, 2009; Ortiz-Ceballos *et al.*, 2019); in other words, they are an essential biological component of sustainable agroecosystems. However, conventional agriculture is frequently criticized for its negative impact on soil quality and human health. A better management of earthworms living in crop fields can solve this problem (Bertrand *et al.*, 2015). In fact, agricultural management studies and programs aimed at increasing soil fertility, degrading organic pollutants, and increasing crop yields, among other things, do not usually take earthworms into consideration.

Before the development of artificial fertilizers and agricultural mechanization, the essential role that earthworms play in agroecosystems had already been suggested by Aristotle, Darwin, and other scientists (Van Groenigen *et al.*, 2019). However, earthworms

are commonly known and referred to as *Eisenia fetida* (used in worm composting or vermicomposting), when in fact the soils of various terrestrial ecosystems and agroecosystems are home to a vast diversity of earthworms (Villalobos-Maldonado *et al.*, 2017). Scientists see earthworms as "ecosystem engineers" as a result of the profound agroecological impact they have on the physicochemical and biological properties of soil (Blakemore, 2009). Most of the studies focused on earthworms have used species adapted to crops, most of which are considered invasive; for instance, in the case of the tropical regions of Mexico and the world, it is very common to find the *Pontoscolex corethrurus* earthworm in conventional agroecosystems —which use fertilizers, herbicides, pesticides, and tillage— and in industrial and urban areas —polluted with heavy metals, crude oil, and others (Taheri *et al.*, 2018; Ortiz-Gamino *et al.*, 2020; Ortiz-Ceballos *et al.*, 2019). In Mexico, the native species *Balanteodrilus pearsei* has a remarkable presence in traditional agroecosystems (Ortiz-Ceballos *et al.*, 2004; Huerta *et al.*, 2005; Fragoso and Rojas, 2014; Fragoso *et al.*, 2015). In this review, written from an agroecological viewpoint, we analyze the largely ignored earthworm services and their potential to encourage agricultural sustainability in Mexico.

Species diversity in Mexico and the world

Earthworms are segmented worms with terrestrial habits. Their lifecycle is synchronized with the soil's temperature and moisture and their length (1 cm-1 m) and width (2mm-3 cm) may vary. They belong to the phylum Annelida, class Clitellata, and order Crassiclitellata (Fragoso and Rojas, 2014). Currently, 5,900 to 6,000 species live in the majority of moist soils in the world; however, it is unlikely to find them in arid and cold areas (Fragoso and Rojas, 2014). There are more earthworm species in Mexico —102 species, equally distributed among native and introduced species— than in several European countries, while in Latin America only Brazil, Colombia, and Ecuador have more species (Fragoso and Rojas, 2014). The most studied tropical regions have been eastern and southeastern Mexico. Veracruz, for instance, has the largest number of known species, followed by Chiapas, Tamaulipas, and Tabasco (Fragoso and Rojas, 2014). The species diversity in tropical agroecosystems is larger than in temperate ones: it varies between four and six species and is rarely larger than ten (Fragoso and Rojas, 2014).

Ecological categories and/or functional groups

Earthworms are grouped in ecological categories or functional groups based on their color, size and body shape, location in the soil, burrowing capacity, and food preference (Bertrand *et al.*, 2015; Van Groenigen *et al.*, 2019). Unpigmented endogeic earthworms build horizontal tunnels in the topsoil and consume soil, organic matter, and soil organisms. Meanwhile, epigeic earthworms live in the soil surface and feed on decaying vegetable material. Finally, the anecic earthworm species are pigmented, they build permanent vertical tunnels or burrows in the mineral layers of the soil, feed on vegetable material, and ingest soil (Bertrand *et al.*, 2015; Van Groenigen *et al.*, 2019).

In Mexico, 92% of earthworms are endogeic (soil feeders). The ten most common species in agricultural soils are: a) introduced species: *Pontoscolex corethrurus*, *Octalasion tyrtaeum*, *Amynthas gracilis*, *Dichogaster bolaui*, and *Aporrectodea trapezoides*; and b) native species:

Phoenicodrilus taste, *Balanteodrilus pearsei*, *Diplotrema murchiei*, *Protozapotecia aquilonalis*, and *Lavellodrilus parvus* (Fragoso and Rojas, 2014). By contrast, the widely known epigeic *Eisenia fetida* ("California red worm") does not dwell in Mexican agricultural soils.

Population size

Earthworm abundance and diversity are determined by crop type, agricultural practices, and the organic residues added to the soil (Manono, 2016). The change in land use also alters the diversity, abundance, and distribution of earthworms. In addition, their survival depends on moisture, temperature, texture, pH, and on symbiont soil microorganisms (Bertrand *et al.*, 2015). Earthworms are therefore assessed for their abundance and biomass. In the tropical regions of Mexico, earthworms have been found in the following type of soils: Arenosols, Fluvisols, Vertisols, Acrisols, Luvisols, Phaeozems, Leptosols, and Andosols. A greater abundance (ind/m^2) of earthworms in these soils has been registered in grasslands (76-704), followed by lands used for coffee (482), cocoa (170), citrus (145), eucalyptus (115), maize (51-222), home gardens (20-110), rain forests (31-170), fallow (298), and others (Ortiz-Ceballos *et al.*, 2005; Huerta *et al.*, 2005; Uribe *et al.*, 2012; García-Pérez *et al.*, 2017).

Soil structure

Earthworms choose mineral and organic particles according to the ecological categories to which they belong (Bertrand *et al.*, 2015; Sánchez-Hernández, 2019). When endogeic and anecic earthworms consume soil for food and to burrow (to build galleries and nests), they mix it in their intestine and produce aggregates (excreta) that contain fragments of the organic matter, organisms, and minerals that were found in the ingested soil. Consequently, earthworm activity contributes to soil formation through the improvement of porosity and aggregation by providing more (Bertrand *et al.*, 2015): a) water flow and retention; b) gas exchange; c) creation of habitats for organisms (microfauna and mesofauna); d) availability of easily absorbed nutrients; e) root growth and carbohydrate release; and f) nutrient absorption for plants.

Earthworm soil ingestion rates are highly variable: 1-30 g of dry soil/g fresh weight/day (Bertrand *et al.*, 2015). Ingestion has a negative relation to age and size: juveniles consume more soil than adults, while smaller ones consume more than larger ones (Bertrand *et al.*, 2015). Therefore, earthworms are capable of regenerating the structure of compacted soils.

Symbiont microorganisms

The most common impact of earthworm activity can be seen on soil microorganisms and it takes place through two compartments: gastrointestinal tract (internal) and movement and feeding (external), associated with the improvement of soil structure (Bertrand *et al.*, 2015, Medina-Sauza *et al.*, 2019; Li *et al.*, 2021). In the first case, some studies have demonstrated the presence of symbiont microorganisms that dwell in the gastrointestinal tract (bioreactor). In addition, earthworm eggs and nephridia (excretory organs) host symbiotic microbiomes (Aira *et al.*, 2018; Li *et al.*, 2021). In the second case, the earthworm mechanical activity modifies habitats and microbial communities that dwell in the soil.

The latter obtain a double benefit from earthworms' movement: they either adhere to their bodies or they are ingested by earthworms, survive, and are subsequently scattered (Aira *et al.*, 2018; Medina-Sauza *et al.*, 2019; Li *et al.*, 2021). Tunnel walls, excreta, and nests are microenvironments with a high microbial and enzyme activity (Van Groenigen *et al.*, 2019). Therefore, the presence of earthworms in the soil promotes a greater abundance of symbiont microorganisms of the phyla Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Planctomycetes, Bacteriodetes, Nitrospirae, and Chloroflexi, that are essential for the survival of earthworms; these microorganisms are capable of degrading organic matter, removing pollutants, releasing easily-absorbed nutrients, and promoting plant growth (Ortiz-Ceballos *et al.*, 2019; Medina-Sauza *et al.*, 2019; Li *et al.*, 2021).

Soil detoxification

Bioremediation has become a usual means to reduce the concentration and toxicity of soil pollutants (Hickman and Reid, 2008; Datta *et al.*, 2016; Villalobos-Maldonado *et al.*, 2017; Sánchez-Hernández, 2019). During the last decade, different studies have pointed out that the impact of the physical (improvement of airing and aggregation), chemical (alteration of pH and OM), and biological activity of endogeic and anecic earthworms on the degradation of crude oil, heavy metals, pesticides, and herbicides, among other things has increased (Hickman and Reid, 2008; Datta *et al.*, 2016; Sánchez-Hernández, 2019). Environmental works that seek to remedy polluted soils do not take earthworms into consideration (Hickman and Reid, 2008; Datta *et al.*, 2016; Sánchez-Hernández, 2019), despite the fact that *P. corethrurus*, *Glossodrilus* sp., *Dichogaster saliens*, *Protozapotecia australis*, and *Onychochaeta windlei* earthworms dwell in soils that have been polluted by the oil industry of Veracruz and Tabasco (Zavala *et al.*, 2013; Hernández-Castellanos *et al.*, 2013a; García-Segura *et al.*, 2018). In addition, toxicological trials have shown that *P. corethrurus* avoids and/or repels concentrations above 10,000 mg of total crude petroleum hydrocarbons (TPH))/kg of dry soil and the median lethal concentration (CL_{50}) of 3,067.3 mg TPH/kg (Cuevas-Díaz *et al.*, 2017). In Mexico, bioremediation studies showed that the presence of *P. corethrurus* and organic amendments reach a 35.6% reduction of benzo(a) pyrene (Hernández-Castellanos *et al.*, 2013b) and 86.0% of TPH (Rodríguez-Campos *et al.*, 2019). Furthermore, earthworms have been found to increase the availability and mobility of essential (Zn, Cu, Mn, Fe) and non-essential (Cd, Pb, Hg) metals in both polluted and non-polluted soils (Taheri *et al.*, 2018). P. corethrurus, for instance, significantly reduced the amount of Pb; additionally, it promotes the Pb phytoextraction capacity of *Lantana camara* (Taheri *et al.*, 2018). Finally, this species tolerates Carbaril (insecticide) concentrations, but Carbendazim (fungicide) and Carbofuran (insecticide) have a lethal effect on it. The response of *P. corethrurus* to herbicides (glyphosate) is variable: the use of glyphosate in coffee crops reduces earthworm abundance and biomass (García-Pérez *et al.*, 2014), but it does not affect reproduction in the laboratory (García-Pérez *et al.*, 2016). Hickman and Reid (2008) and Datta *et al.* (2016) have therefore suggested that endogeic earthworms can be used as indicators of agricultural, industrial, and urban soil health, as well as to restore degraded soils.

Nutrient availability

Since they consume and degrade a vast diversity of organic materials, earthworms directly affect soil fertility, increasing total OM (40-48%), total P, and total N concentrations (Van Groenigen *et al.*, 2019). This is the result of biochemical transformation processes that occur in the digestive tract of earthworms and that involve: a) production of endogenous cellulases (Ean-Eg, EF-EG2, and GHF9); b) association with intestinal microbiota (Protobacteria, Firmicutes, Actinobacteria, Chloroflexi, and Bacteroidetes); c) gene expression (transcriptome) that adjusts their digestive system (salivation, gastric acid, and pancreatic secretion); d) improvement of the digestive efficiency based on caecum type; and e) association with nephridial bacteria (Pedobacter) (Ortiz-Ceballos *et al.*, 2019). It has thus been demonstrated that total element concentration substantially increases in earthworm excreta: 241% and 84% for mineral N and available P, respectively (Van Groenigen *et al.*, 2019); moreover, there is a significant increase in pH (0.5 units), in cation exchange capacity (40%), and in base saturation (27%). In addition, earthworms accumulate, store, and recycle carbon and nitrogen in their biomass. They have a low carbon assimilation efficiency (2-27%; maximum: 70%), depending on the species, density, organic matter quality, and temperature. Nonetheless, the amount of nitrogen circulating through their biomass is higher than carbon —the estimated values fluctuate between 60 and 100 kg/ha/year—, which suggests an important flow of this nutrient in agroecosystems. Therefore, an appropriate management of organic residues or management practices (cover crops, intercropping, crop rotation, and no-till farming or minimum tillage), can maintain abundant earthworm populations, in order to synchronize the release of easily absorbed nutrients that will then be available for plants when they need it. This suggests the need to design and improve agroecosystems that maintain soil fertility to achieve a sustainable crop production (Bertrand *et al.*, 2015) —for instance cocoa, coffee, maize, and other agroecosystems in which the farmers of southeastern Mexico are involved (Brown *et al.*, 2004; Ortiz-Ceballos *et al.*, 2005; Huerta *et al.*, 2005; Juárez-Ramón and Fragoso, 2014; Fragoso *et al.*, 2015; García-Pérez *et al.*, 2017). Finally, earthworms are soil improving agents that cannot be studied separately from agroecosystems (Van Groenigen *et al.*, 2019) (Figure 1).

Earthworm-plant-insect interaction

Almost nothing is known about the effects of soil organisms on a vast range of natural enemies: predators and parasitoids (Wurst, 2013; Heinen *et al.*, 2018; Thakur *et al.*, 2021). Although conceptual frameworks are well developed and some interactions are well studied (*e.g.*, mycorrhizae), gaps still exist regarding the biodiversity knowledge about other taxa that are closely associated with plant roots, such as earthworms (Shelef *et al.*, 2019; Li *et al.*, 2021). Soil organisms play a significant role in the configuration of plant-insect interactions in the field, with general patterns for some taxa, such as earthworms (Heinen *et al.*, 2018). Results show that plants use earthworm excreted nutrients and assimilate them into their roots, leaf tissue, and phloem sap, which is sucked by aphids (Shelef *et al.*, 2019; Thakur *et al.*, 2021). This, in turn, shows that earthworms in the soil and aphids on the plants are functionally interconnected. Earthworm presence increased the production of the plants'

Figure 1. Effects of the native tropical earthworm *Balanteodrilus pearsei* on the growth of the corn crop. Trays with labels: Green=Soil+Legume Foliage+Earthworm; Blue=Soil+Legume Foliage; Red=Soil+Earthworm; Yellow=Soil+Earthworm.

chemical defenses against cell feeders (trips) by 31% and their resistance against trips by 81%. Both results were associated to earthworm abundance and diversity (Xiao *et al*., 2017). Results suggest the need of a better integration of soil fauna into plant-herbivore interaction studies, both in applied and basic research. This opens up opportunities to explore the manipulation of soil organisms in agriculture or ecosystem restoration. Some groups can be promising agents for the improvement and protection of crop yield and can affect plant diversity on the soil at a community level, which allows the use of soil organisms to guide vegetation development (Wurst, 2013; Heinen *et al*., 2018) (Figure 2,3,4,5).

Plant growth and productivity

Epigeic, endogeic, and anecic earthworm activity has an important beneficial effect on plant growth and productivity because it: a) provides a greater amount of nutrients; b) improves soil structure; c) stimulates microbial symbionts of plants; d) controls pests and illnesses; and e) produces plant growth regulating substances (Ortiz-Ceballos *et al*., 2007; Van Groenigen *et al*., 2014). During their assessment of the three main basic crops (corn, wheat, and rice) and pastures in 58 studies conducted in almost every continent—

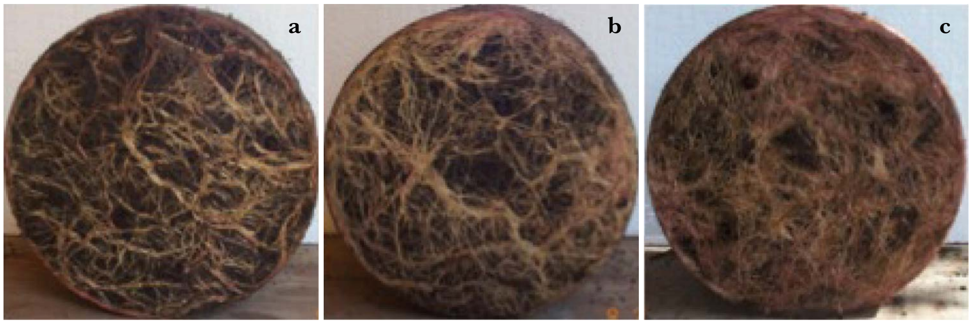

Figure 2. Effect of the native tropical earthworm *Balanteodrilus pearsei* on the growth of the roots of the corn crop: a) Soil+Earthworm; b) Soil+Legume Foliage; c) Soil+Earthworm+Legume Foliage.

Figure 3. Construction of galleries and production of excreta of the native tropical earthworm *Balanteodrilus pearsei*.

Figure 4. Exotic tropical earthworm *Pontoscolex corethrurus*.

Figure 5. Construction of galleries and production of excreta of the exotic tropical earthworm *Pontoscolex corethrurus*.

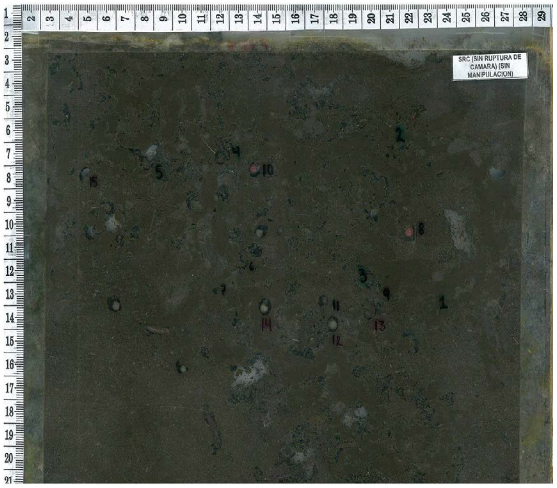

Figure 6. Cocoon and excreta production of the endogenous earthworm *Pontoscolex corethrurus*.

save in Antarctica—, Van Groenigen *et al.* (2014) determined that earthworms significantly increased crop yields (25%), aerial biomass (23%), roots (20%), and total biomass (21%). The most important effects on grain production are achieved with a >30-40 g fresh weight/m^2 biomass (Van Groenigen *et al.*, 2014). Nevertheless, ecological, and agricultural studies have been conducted for the handling of only 11 out of 102 species in Mexico. Among them *P. corethrurus*, *Polypheretima elongate*, and *B. pearsei* stand out as a result of their potential *in situ* use in agroecology and/or in regenerative agriculture (Fragoso and Rojas, 2014). For instance, the presence of *B. pearsei* and *P. corethrurus* earthworms with the velvetbean legume (*Mucuna pruriens* var. *utilis*) significantly increased the growth and production of corn, vanilla, and Roma tomato crops (Ortiz-Ceballos *et al.*, 2007); moreover, used in nurseries, they boosted the growth of *Abies religiosa* (Sánchez-Velásquez *et al.*, 2019) and *Quercus insignis* forest seedlings (Avendaño-Yáñez *et al.*, 2017). However, domestication has turned hundreds of traditional plant species to intensive farming (Porter and Sachs, 2020). Farming practices and intense selective breeding used to increase yields can involve a hidden cost: the interruption of interactions between plants and earthworms (Porter and Sachs, 2020). It is therefore suggested that, in order to improve a sustainable agricultural production, agroecological research must develop methods to optimize relations between cultivated plants and soil organisms (Porter and Sachs, 2020).

CONCLUSIONS

Over the last 150 years, the relevance of earthworms and their evolution with domesticated plants have been attested (Braga *et al.*, 2016). Therefore, it is very likely that earthworm services contribute to increase agricultural sustainability improving human health (Bertrand *et al.*, 2016). This suggests the need of long-term field studies about the impact of earthworms on crop production. In addition, agricultural grants and funding are required to design and promote agroecosystems that take into account the intrinsic value of earthworms and the effect of crop practices on earthworm diversity, abundance, and activity (Bertrand *et al.*, 2015; Scown *et al.*, 2020).

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AP AGRO PRODUCTIVIDAD

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Success and resilience factors of rural enterprises in Mexico: RENDRUS case

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ABSTRACT

Objective: To identify the success and resilience factors of rural enterprises in Mexico in order to contribute to the theoretical and empirical differentiation of these concepts.

Design/Methodology/Approach: We used the National Network for Sustainable Rural Development Companies as a case study to formulate key questions to identify their success and business resilience factors.

Results: Permanent job creation turned out to be the main success factor, and timely delivery of the product was the main resilience factor.

Study Limitations/Implications: Recognizing the difference between success and resilience factors can help rural enterprises, their leaders, and decision-makers in the rural sector to understand and adjust their operating strategies based on their objectives.

Findings/Conclusions: A success factor is a way to stand out among similar rural enterprises, while a business resilience factor serves to face adversity and continue over time.

Keywords: success, failure, agribusiness.

INTRODUCTION

Enterprises are a fundamental pillar of the economy as they are centers for the generation of economic resources and value (Pavón, 2010). In 2018 there were 4,057,719 enterprises in Mexico, out of which 97.1% were microenterprises, 2.7% were small and medium-sized, and 0.3% were large (INEGI, 2018); the rural enterprises of this study are found in the first category. According to Arvelo (2004), the rural enterprise is an organization located in the rural sector whose purpose is to generate profits from the use of the factors and capitals of its territory.

Rural enterprises are not exempt from recurring problems that place them in a situation of low survival. In this regard, Pavón (2010) indicates that in 2008, only 17.5% of Mexican enterprises survived and the other 80% of these enterprises closed down definitively before reaching their first year of operation.

The word "success" of an enterprise is a very broad and subjective concept; Luk (1996) mentions that a company can be successful when the owner or manager has obtained a surplus in profits. The literature identifies success factors with different approaches, including natural leadership, responsibility and honesty of the leaders, permanent training, adequate financing, and elimination of intermediaries (Valtierra *et al.*, 2008); the business plan, management, operational procedures, and conflict resolution (Avendano-Alcaraz *et al.*, 2009); available technology, the offer of more than one product, and high market integration

RENDRUS
RED NACIONAL DE DESARROLLO RURAL SUSTENTABLE

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(Remilien *et al.*, 2018); the degree of schooling, the number of years in productive activity, trust in government institutions and relational capital (Jaramillo *et al.*, 2013).

Valtierra *et al.* (2008) indicate that the factor that leads to success in more than 75% of rural economic organizations is for the organizational process to be truly assumed by its members. The success factors of an enterprise are not necessarily the same as the resilience factors.

Resilience is an ability to surface from adversity, adapt, recover and access a meaningful and productive existence (Kotliarenco *et al.*, 1997). In turn, Meneghel, Salanova and Martínez (2013) point out that business resilience is composed of factors originating from critical or adverse situations, resulting from the weaknesses and threats faced by the enterprises.

Theory states that any significant disruption has consequences on the enterprise's performance, whether in sales, production level, customer service or other (Sheffi & Rice, 2005). It also states that the disruptive situation faced by the company has eight phases (Sheffi & Rice, 2005); in this regard, Sanchis and Poler (2011) explain that it would be valuable to be able to identify the factors of business resilience even before the preparation phase of the enterprise. This study addresses the need to identify the factors that make the company resilient.

The concept of resilience used in this study refers to the capacity of rural enterprises to face adversities, crises and uncertainties, through their risk and protective factors, in a given period, so that changes and ruptures can be generated to prevail over time (Cordero *et al.*, 2014).

The objective of this study was to analyze the factors of success and business resilience of rural enterprises in Mexico, through their identification, with the purpose of contributing to broaden these concepts and to understand the actions that contribute to the survival of the enterprises. The hypothesis underpinning the present study was that the success and the resilience of rural enterprises are connected because there may be factors that contribute to the success of rural enterprises and coincide with factors that contribute to business resilience. However, they are different concepts that should not be expressed as synonyms.

MATERIALS AND METHODS

The objective of the study were the 1,278 rural enterprises of the National Network for Sustainable Rural Development (*Red Nacional de Desarrollo Rural Sustentable*, RENDRUS) during the period 2004-2010, located throughout the country. The base was generated from information of the enterprises at www.rendrus.gob.mx that was available until 2015, where the leaders of the rural enterprises (owners or legal representatives) expressed the success factors of the enterprises they represent. The research was qualitative. The critical success factors methodology proposed by Romero *et al.* (2009) was used, which is characterized by the collection of information through questions. The success of an enterprise was identified through the question: What has made your company different to generate more profits or to differentiate its product or service? The question was formulated based on Luk's (1996) definition of success. The resilience was identified through the question: What action did the company have to implement to face certain adversity that allowed it to continue through time and be in a better situation? The question was formulated in this way to identify the resilience factors of the rural enterprise (Sanchis & Poler, 2011). Finally, the proportion of the presence of business success and resilience factors was calculated for the total number of enterprises.

RESULTS AND DISCUSSION

The enterprises studied were classified into three types according to their characteristics.

Data analysis consisted of a list of 178 success and resilience factors of the companies under study, which were classified into 116 success factors and 99 resilience factors. The success factors of the companies studied are shown in Table 2. The percentages indicated in the tables represent the proportional share of each factor in the companies studied, showing the top ten.

A success factor is determined by the benefits it generates. The main factor found in rural enterprises that participate in the RENDRUS is permanent self-employment. Remilien *et al.* (2018) point out as one of the main factors for the creation and permanence of microenterprises that people have no other work option, and these are known as entrepreneurs out of necessity.

In the type of organization of the rural enterprise, informal groups stand out, which are established by friends, family, and temporary workers. According to Hernández (2007), the family system plays a major role in the performance of the enterprise, as a result of the permanent search to guarantee the family's well-being and economic stability.

Another success factor identified is having a high production volume, resulting from high yields or greater product generation. In this regard, Belausteguigoitia (2003) suggests that the focus on productive aspects is a common characteristic of family businesses, as they tend to be oriented towards production and sales.

Profit is considered as an indicator that influences the success of rural enterprises, the representatives of the enterprises express that the economic activity must generate profits for the family or for the partners. However, this result is opposite to what was analyzed

Table 1. Classification and characteristics of the rural enterprises studied.

Rural Microenterprise A	Rural Microenterprise B	Rural Microenterprise C
Informal and family microenterprise, more than ten years of existence, less than 5 members. The business has basic services. Few tools and without information and communication technologies (ICTs). It has used financing. Does not receive training in a continuous manner. There is trust between members of the enterprise and the suppliers. Strengths: discipline and honesty. Lack of economic resources to invest in labor capital. They reinvest 60% of income or less.	Informal and family microenterprise, with more than five years of existence. Between 1 and 3 employees. Direct sale to the local consumer. Does not have the necessary infrastructure to work, has basic services, sufficient tools and equipment. Low technological level since there are no ICTs. Never has had access to financing or training. Strengths: good customer care and product quality. Lacks economic resources to invest in labor capital.	Family microenterprise, of 6 members or more. Direct sale to the local consumer. It has basic services, without access to ICTs, financial resources or training. The members of the family do not receive a salary for their work, 70% of their income is reinvested in the activity. It is a disciplined group in work. Lacks economic resources for labor capital. Good quality product. It does not have product variety, price according to production costs. Without new market options.

Source: Prepared by the authors with information from www.rendrus.gob.mx

Table 2.

Success Factor	Definition	Percentage
Permanente self-employment&	Giving work to someone to conduct an activity permanently, including the owner of the Enterprise.	10.3
Type of organization&	Social system designed to achieve goals and objectives through human resources or talent management.	8.6
High production*	Having high yields in its production, whether agriculture and livestock, or agriculture and industry.	6.6
Temporal employment&	Employing someone in the performance of a productive activity seasonally.	6.2
Profit$	It is the net benefit obtained by the capital invested in the enterprise.	5.4
Benefit generated$	It is the economic and social benefit obtained by the establishment of the enterprise.	3.2
Experiencia*	Form of knowledge or ability derived from observation, housing or from practice.	3.1
Capacity installed*	Generating a maximum production volume during a specific period, considering the local resources.	3.1
Quality*	Set of properties inherent to a product or service to satisfy implicit or explicit needs.	3.1
Acceptance of the product$	Acceptance of the product or service and amount that it is willing to acquire.	3.1

Note: Factor classification: &Organizational, $Economic, *Productive.
Source: Own elaboration with information from www.rendrus.gob.mx

by Remilien *et al.* (2018), where they compare rural micro-entrepreneurs against normal entrepreneurs, and found that the former aim at survival and the latter aim at profit. The main resilience factors identified are shown in Table 3.

The elements indicated in Table 3 are considered as resilient because they are the skills and capacities that the enterprise has and that allow it to remain in the long term meeting its economic, social and environmental objectives.

Table 3. Main resilience factors of rural enterprises (2004-2010).

Resilience Factor	Definition	Percentage
Timely delivery&	Fulfilling the time agreed with the delivery of products and services.	16.8
Effective commercialization &	Way in which they trade the product: individual (even going to the address), collective (in association with another enterprise), or both.	12.1
Productive capacity*	It is the maximum level of activity that can be reached with a productive structure within the enterprise.	9.6
Broad client portfolio&	Having a broad number of clients.	6.6
Professional management&	The person that leads the enterprise has an adequate professional profile to develop this activity.	5.5
Biotechnology*	Application of new technologies in biological processes or agricultural production.	3.4
Group discipline&	Each of the members of the enterprise respects a schedule, hierarchical levels, and activities.	3.3
Exports$	Selling the products generated to other countries. The enterprises that have a market outside, which are considered a competitive advantage.	3.3
Distribution&	The way in which products are taken from the production unit to the final consumer.	2.7
Support received$	Sources of help for something to happen.	2.6

Note: Factor classification: &Organizational, $Economic, *Productive.
Source: Own elaboration with information from www.rendrus.gob.mx

In the case of the RENDRUS enterprises, timely delivery was identified as the main resilience factor. This finding coincides with that analyzed by Sanchis & Poler (2011) who report how timely delivery impacted an increase in market share from 27% to 30%. Therefore, it is advisable to adopt strategies that take into account production parameters, timeliness and delivery of the product or service to meet customer expectations and face the competition.

Partnering with other companies to market the product allows to decerase marketing costs and cover larger-scale orders. In this regard, Rubio and Aragón (2008) state that one of the sources of competitive success is commercial resources such as access to markets and distribution channels, so companies must respond quickly to trends.

Businesses have had to expand their production capacity to meet unsatisfied demand and abide by their customers. Production capacity is the result of the decision to increase market share; it is a productive and technological decision (Tapia, 2012). The resilience factor "productive capacity" is related to the success factors of "high production" and "own installed capacity"; these factors generate strength in the company to respond to such demand.

Professional management refers to the fact that it is desirable for the administration of the company to be entrusted to a professional person, because of his or her ability to make appropriate decisions. However, professionalization is a process of gradual transformation that implies a change of mentality in each of the members of the enterprise (Belausteguigoitia, 2003).

Biotechnology is defined as "any technological application that uses biological systems and living organisms or their derivatives to create or modify products or processes for specific uses" (SCDB, 2018). The use of agricultural biotechnology is a resilience factor because it strengthens rural enterprises, since it has an impact on the quality and level of production; in addition, in order to use it, it is necessary to receive training, thus strengthening the enterprise with technological knowledge.

Finally, to compare a success factor and a business resilience factor, the following definitions are set out in Table 4.

As can be seen in Tables 2 and 3, the hypothesis that the success factors were not the same as the resilience factors for the rural enterprises studied is proven; however, a similarity was found between high production, own installed capacity, and productive capacity. In addition, there are resilience factors that contribute to the success of the enterprises, as pointed out by Rubio and Aragón (2008) referring to marketing, and other factors that

Table 4. Success factor and resilience factor of rural enterprises.

Success Factor	Resilience Factor
Internal and external factor that responds to a predefined standard by a development model that influences the enterprise positively, placing it as reference for other enterprises, since it presents singular characteristics that allow it to be different from others (SAGARPA, 1996).	Element that promotes the capacity to face critical situations, through the recognition of risk and protection factors of the enterprise, which allows absorbing changes, ruptures and prevailing in time (own definition).

Source: Prepared by the authors.

were considered by Gibb and Ritchie (1982) as necessary to have a successful start were motivation and determination. Finally, we find that Morales-Jiménez *et al.* (2015), in their study with rural microenterprises, found that schooling is a success factor for enterprises, which is related to professional management.

The results serve as a reference to generate strategies that help the business sector to make decisions that help to prepare the company to face disruptive events or critical situations, thus achieving resilience. It was identified that the greatest number of critical situations occurred in economic, productive and organizational aspects, so it is in these areas where more resilience factors have been developed, as indicated by López *et al.* (2011). It is important to note that an enterprise can be resilient and also successful, which is the best-case scenario. However, although business success and resilience are related attributes, a company does not always have both characteristics.

CONCLUSIONS

There is an important difference between a business success factor and a business resilience factor, and it lies mainly in the fact that success is a way of standing out among similar companies, while a business resilience factor serves to face adversities and continue over time.

The success factors are mainly economic and productive, as opposed to the resilience factors, which are mostly organizational. With these results, the hypothesis set out was confirmed. Identifying both factors can help rural enterprises to understand and adjust their strategies for permanence despite adversities. Permanent self-employment, type of organization and high production were the main success factors of the enterprises studied, while customer service, effective marketing and productive capacity were the main resilience factors.

Some open lines of research are to verify whether it is possible for an enterprise to be successful but not resilient and vice versa; furthermore, with the factors identified, it is possible to propose a scale for measuring resilience in rural enterprises.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Detection of huanglongbing of citruses (*Citrus* sp.) through Sentinel-2 satellite images in Huimanguillo, Tabasco, Mexico

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ABSTRACT

Objective: Develop a fast, practical, and inexpensive methodology for determining plants with HLB symptoms by exploring the potential use of Sentinel-2 satellite images.

Design/Methodology/Approach: Sentinel-2 images were used to perform a supervised classification to discriminate healthy Persian lime trees and trees with HLB molecularly verified by qPCR, and other land uses in the citrus growing area of Huimanguillo, Tabasco.

Results: The results showed that in the green (560nm), red (665 nm), and near-infrared (705 nm) band regions, the spectral response of trees with HLB is higher than that of healthy trees. Likewise, there is an area of 26.96 and 12 981 hectares of Persian lime affected and free of HLB, respectively, with an accuracy of 0.84 % and a Kappa coefficient of 0.83. Field verification corroborated three plantations where plants with HLB were previously detected and are currently in production.

Study Limitations/Implications: The study was carried out in the season of less cloud cover, as this is a limiting factor for the image analysis since it can lead to inadequate tracking of the affected area.

Findings/Conclusions: The results showed that it is possible to differentiate Persian lime plants affected by HLB from healthy plants using Sentinel-2 satellite images.

Keywords: Citrus greening, *Candidatus* Liberibacter asiaticus, satellite image, spectral reflectance.

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INTRODUCTION

According to data from the Servicio de Información Agroalimentaria y Pesquera, in 2020 the surface cultivated of citrus in Mexico was approximately 572 017 hectares, which generated an estimated production of 8 017 277 tons of fresh fruit. Of this cultivated surface, 58% is occupied by the orange, 21% by Persian lime, 17% by Mexican lime, and 4% by grapefruit and pomelo. In the state of Tabasco, there is a cultivated surface of 15 501 hectares of citrus, of which 94% are established in the municipality of Huimanguillo, with 14 189 ha of Persian lime and 438.5 ha of Valencia orange, contributing a production of 171 966 tons of fresh fruit (SIAP, 2020); however, there are adverse factors such as pests and diseases that reduce their quality and production. Globally one of the most critical diseases today is citrus huanglongbing (HLB), considered one of the limiting factors in production since it reduces it in most cultivated areas, million citrus plants have been infected, and the yield has decreased (Bové, 2006). In the case of Mexico, HLB is caused by the bacteria *Candidatus* Liberibacter asiaticus (CLas), restricted to the plant's phloem; the bacteria is transmitted by the Asian citrus psyllidae, *Diaphorina citri*. In México, the first detection of CLas was carried out in 2009 in Yucatán (Trujillo-Arriaga, 2009), and in 2012 it was reported in Tabasco (Trujillo-Arriaga, 2012) after having been reported in other states of the north and south of the country. Currently, CLas and *D. citri* are distributed in all the citrus zones of the country. The productive losses that have been reported due to this disease have been 62% in Mexican lime (Robles-González *et al.*, 2013) and 17% in Persian lime (Flores-Sánchez *et al.*, 2015). The characteristic symptom is a spotting of irregular and asymmetrical edge with regards to the central nerving of the leaves. In some cases, in the tree affected, there are yellow outbreaks with a sectoral distribution (Halbert and Manjunath, 2004; Gottwald *et al.*, 2007). Once a tree branch is infected, the bacteria propagate gradually on the entire tree. The timely detection and elimination of sick trees are recommended to avoid the dispersion of HLB and monitor the plantations affected and control the vector insect (Keremane *et al.*, 2015). The visual inspection of the trees by trained personnel is one of the most frequently used ways of detecting trees with HLB symptoms (Keremane *et al.*, 2015); however, presently the most efficient method for the detection and confirmation of HLB is based on molecular detections through the use of the Polymerase Chain Reaction test in real-time (qPCR) (Hansen *et al.*, 2008). However, both activities require a high investment of economic resources. In this sense, remote sensing emerges as a valuable and inexpensive tool for detecting different biological characteristics of the crops since data from the crops can be compiled quickly and analyzed in real-time. Recently the use of Sentinel-2 satellite images has proven to be efficient for the identification of damage in crops provoked by different pathogens, such as the corn streak virus (Dhau *et al.*, 2019), where it was found that the maize infected was highly separable from the healthy maize and other classes of land-coverage with a general accuracy of 85.29%. Caasi *et al.* (2020) evaluated the use of an economical method to detect damage from a bacterial blight on rice leaves and found that the sensors of the Sentinel-2 satellites have the potential of detecting and estimating the damages caused by the bacterial rice blight; likewise, Liu *et al.* (2020) implemented a spectral index based on the infrared region to detect the blight caused by *Fusarium* in wheat, and the general accuracy obtained was 78.6%. Therefore, once the possible areas affected are detected, the producers can apply the necessary measurements in those zones without the need to inspect the entire cultivated surface. Thus, this study aimed to explore the potential use of Sentinel-2 satellite images to identify Persian lime trees with HLB symptoms and differentiate them from healthy trees in the citrus zone of Huimanguillo, Tabasco.

MATERIALS AND METHODS

The study area includes the citrus zone in the municipality of Huimanguillo, Tabasco (Figure 1). It is located between the extreme geographic coordinates: 17.95° and 17.57° latitude north, and −93.43° and −93.76° longitude west.

Figure 1. Study area, citrus zone of Huimanguillo, Tabasco.

Field sampling and data obtaining

Field sampling was conducted in two Persian lime plantations (PL) of 25 and 17 hectares, respectively (identified as plantation 1 and 2), which had an official report of cases of positive plants to CLas through the qPCR test, performed as part of the activities of the phytosanitary program against regulated citrus pests by the Plant Health State Committee (*Comité Estatal de Sanidad Vegetal de Tabasco*, CESVETAB). For the observation of symptoms, sampling was directed at plants with positive detections for the verification and georeferencing of each of the plants positive to CLas.

Image acquisition

Sentinel-2 satellite images, which have a resolution of 10 m, were obtained for free from the USGS Global Visualization Viewer (GloVis) page, with the date March 26, 2020. The images were downloaded as compressed GeoTiff files, with UTM projection zone 15 and datum WGS84, and later they were integrated into a geographic information system managed with Quantum GIS (QGIS) version 3.16. Based on the polygons from the citrus plantations present in the zone, which are available in the CESVETAB database, the delimitation of the citrus zone was carried out to cut the images and adjust them to the study area. Later, for the following processes, the Semi-Automatic Classification Plugin (SCP) complement was used, which is a plugin for QGIS that allows the semi-automatic classification of remote sensing images (Congedo, 2020). The atmospheric correction was done using the automatic algorithm of the complement and the union of the 13 bands in a single virtual image to ease manipulating the information and maintain the most significant amount of helpful information.

To carry out the classification, a combination of 4-3-2 bands with natural color was used, with which the creation of samples or training sites was done, taking as reference the previous knowledge from the zone, to discriminate PL trees with HLB from healthy PL trees and the other coverages in the zone. For each type of coverage, 10 training areas were selected, and a supervised classification was made through the minimal distance algorithm.

For the validation of the classification, field verification was made of the plantations with possible HLB, healthy plants, plantations different from the training areas, and the polygons of citrus plantations were used as support. For the verification of the classification, r.kappa algorithm was used to calculate the kappa coefficient to evaluate the accuracy of the classification result. Values >0.80 represent a strong agreement or accuracy between the classification map and the terrain's reference information; values between 0.40 and 0.80 represent moderate agreement; and values <0.40 represent scarce agreement (Jensen, 2015).

RESULTS AND DISCUSSION

Based on spectral signatures obtained from the satellite image through the supervised classification, eleven classes were generated: PL plantations of less than five years and six years or more with possible HLB; PL plantations of three years or less; PL of five, eight, and fifteen years. These classes were created because, in the citrus zone, there are plantations with patches of different ages due to sowing and re-sowing of replacement seedlings. Likewise, or alternatively, there are plantations combined with other crops; however, in the classification, plants of less than 5 years of age were grouped as PL1 with HLB and plants of 6 years or more as PL2 with HLB; healthy PL of ages: less than 5 years, 5 to 10 years, and more than 10 years. The other classes of land coverage identified included grasslands, secondary vegetation, bodies of water, bare soil, and urban areas (Figure 2).

Similarly, the surface occupied by each of the classes was calculated: PL1 with HLB of less than five years, 24.44 ha; PL2 with HLB older than six years, 2.52 ha; PL less than five years, 6086.68 ha; PL of five to ten years, 4848.49 ha; PL older than ten years, 2045.95 ha. In total, the surface obtained was 26.96 hectares of PL affected by HLB and a surface of 12 981 ha free of HLB, which in total integrated a surface of 13 008 ha, indicating an incidence of HLB of 0.2%, which is considered a low incidence with regards to the total of the surface cultivated in the zone. Regarding the plantations with official positive detections, the incidence found through the classification is between 4.6 and 12% in plantations 1 and 2, respectively.

Figure 3 shows the spectral signatures of the PL trees with HLB and the healthy trees. It can be seen that in the regions of the green (560nm), red (665 nm), and near-infrared band (VNIR) (705 nm), the spectral response of the sick trees is higher than those of the healthy trees.

This result agrees with what was found by Mishra *et al.* (2009), who studied the spectral characteristics of the leaves infected with HLB in visible and near-infrared regions, they found that the green (530-595 nm) and red (710-750 nm) bands in the visible region have an excellent potential to discriminate HLB. Similarly, Mishra *et al.* (2011) used the visible bands at 570 nm and 670 nm in a multispectral sensor in drones to detect the citrus trees infected with HBL in field conditions and achieve accuracy higher than 95% in the classification.

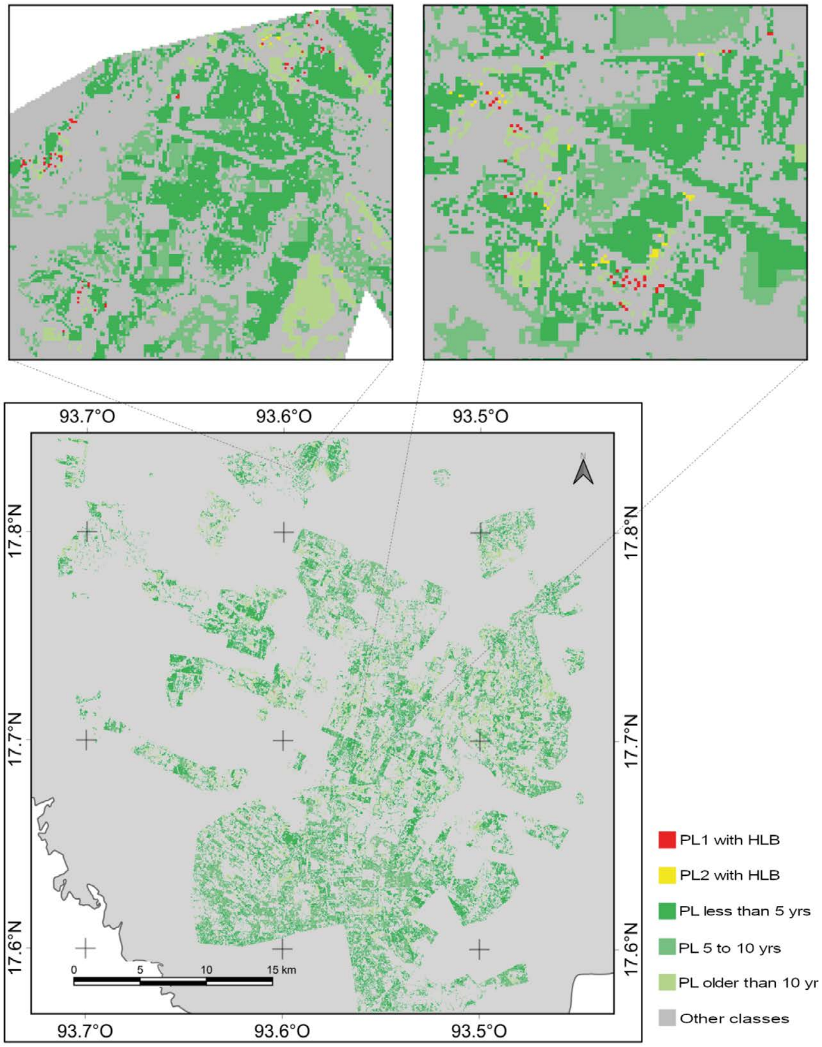

Figure 2. Image classified from the citrus zone in Huimanguillo, Tabasco 2020. The zones with healthy Persian lime and with HLB are represented.

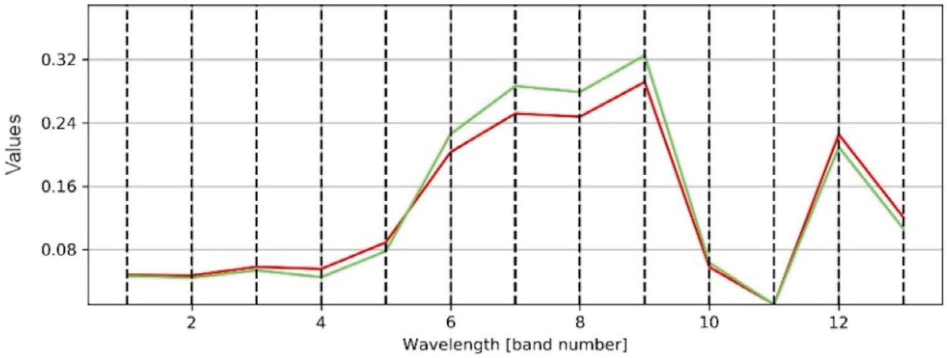

Figure 3. Spectral signatures of trees with HLB in red and healthy trees in green.

In the field validation, it was found that there are six plantations with HLB symptoms, of which three already had detections through qPCR for HLB and which currently still maintain production; however, the highest amount of plants affected were found in the central and northwest part of the citrus-producing zone with an incidence of up to 12% in one of the plantations visited, which is why it is imperative to emphasize that zone with the purpose of limiting greater infection, taking into account that Yzquierdo-Alvarez *et al.* (2021) reported that the space-time fluctuations of *D. citri* in such a zone presents high populations, increasing the risk of dispersion towards neighboring plantations.

The sampled positive plantations detected presented plants with typical visual symptoms caused by HLB (Flores-Sánchez, 2015). These results indicate that it is possible to differentiate the PL plants spectrally with HLB from healthy plants using Sentinel-2 satellite images since the canopy of the trees sick with HLP has higher reflectance in the visible range than the canopy of the healthy trees (Sankaran *et al.*, 2013).

In the classification performed, there was an accuracy of 0.84% and Kappa coefficient of 0.83, indicating a strong classification (Jensen, 2015). The accuracy found in this study is higher than that reported by Li *et al.* (2015), where the differentiation of HLB was conducted through satellite images, WorldView-2 to monitor HLB quickly in large citrus plantations and to evaluate the capacity for HLB detection, and they obtained an accuracy of 81% and Kappa coefficient of 0.464, a value lower than that found in our classification. Likewise, Sankaran and Ehsani (2011) found that HLB was detected in citrus leaves with a classification accuracy of the average sick class of approximately 75 to 84%, which corroborates that using Sentinel-2 images can be a helpful tool for the timely detection of plants with HLB symptoms in extensive areas.

Adopting the methodology resulting from this study optimizes the search for trees with HLB symptoms by requiring a lower investment of personnel, time, and economic resources in comparison to the conventional visual inspection, at the same time that this increases the accuracy of detection, since in order to identify a tree infected with HLB through visual inspection the accuracy is between 47% and 59% (Futch *et al.*, 2009).

It should be mentioned that in this study, the nutritional state of the trees was not contemplated, nor the period of symptom observation time, the management to which the plantations are subjected, the phenology of the crop, or the environmental factors, which is why these factors could be of use in a future classification and possibly improve the results found here.

CONCLUSIONS

The classification performed differentiates Persian lime trees with HLB from healthy trees with an accuracy of 0.84%, which is why using Sentinel-2 satellite images can be used as a useful tool for the timely detection of plants with HLB symptoms and to streamline plant health intervention processes to minimize the regional dispersion of HLB.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of indole-3-acetic acid on vegetative propagation by cutting cuatomate (*Solanum glaucescens* Zucc.)

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ABSTRACT

Objective: To evaluate the effect of indole-3-acetic acid and budwood type in the vegetative propagation by cutting cuatomate (*Solanum glaucescens* Zucc.) and to describe the phenological stages, in order to increase production.

Design/Methodology/Approach: A completely randomized design with a 22 factorial design was used to estimate the effect of two concentrations of indole-3-acetic acid (1000 and 10000 ppm) and budwood type (secondary and tertiary) on the number of leaves and sprouts of the plant. With regard to propagation, 30-cm budwoods from secondary and tertiary branches of cuatomate were used; transversal and diagonal cuts were made at the ends of the branches that generate the canopy and the root, respectively. Data was subject to an analysis of variance, using the general linear model procedure.

Results: Highly significant differences ($p \leq 0.01$) were observed between the variables under study. Higher number of leaves and sprouts (16.700 and 20.000, respectively) were observed in tertiary budwoods inoculated with 1000 ppm of IAA. In the evaluation of the phenological stages, the first bud appeared at 30 days, while the first leaf and flowers appeared 40 and 180 days after inoculation, respectively.

Study limitations/implications: Commercial candelilla wax was applied in the transversal cut to prevent attacks by pathogens and every single budwood was completely covered with indole-3-acetic acid.

Findings/conclusions: The use of indole-3-acetic acid in the vegetative propagation of cuatomate would be an alternative to increase its production; promoting a sustainable activity in the Mixtec region of Puebla.

Keywords: Auxin, wild plant, Mixtec

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INTRODUCTION

Mexico has an abundance of genetic resources; its genetic information has actual or potential value and utility which forms the basis of humanity, fulfills basic needs, and contributes to counteract hunger and poverty (Estrella *et al.*, 2005; Martínez-Pérez *et al.*, 2012). The Low Mixtec region of Puebla is located in the south of the State of Puebla, Mexico; it is characterized by its warm dry or semi-dry climate. Its poor soils and rugged orography make it one of the most marginalized areas of the country (Guízar-Nolazco *et al.*, 2010; Hernández *et al.*, 2011). The

inhabitants of the region consume a great variety of wild plants. Cuatomate (*Solanum glaucescens* Zucc.) is a wild plant on the process of domestication; it has a high economic, ecological, and nutritional potential and is primarily used for human consumption in the Low Mixtec region of Puebla, Mexico (Gutiérrez-Rangel *et al*., 2011; Medina-Galicia *et al*., 1996). Mixtec farmers identify three types of cuatomate, depending on the external color of the fruit (from its early stages of development to the beginning of its ripening): green, white or ashy, and mottled (Gutiérrez-Rangel *et al*., 2011). Cuatomate, as a phytogenetic resource, is closely related to the traditional lore (farmers' lore); therefore, it is widely used, has great value and specific functions (Martínez-Pérez *et al*., 2012). Its fruit is used to prepare sauces and stews, replacing tomatoes and tiny green tomatillos; according to the inhabitants of the region, this fruit has an exquisite and irreplaceable taste (Hernández-Rojas *et al*., 2018). The transportation, marketing, and promotion of cuatomate in the United States by Mixtec emigrants has led to an increasingly unsatisfied demand (Gutiérrez-Rangel *et al*., 2011; Hernández-Rojas *et al*., 2018). This situation has contributed to the acceleration of domestication. However, this practice indirectly promotes the deterioration and disappearance of cuatomate in its natural habitats, since this crop method involves taking plants away from their natural environment. Therefore, alternatives aimed at the use of the species in a sustainable agriculture framework are required. There are few studies about cuatomate; however, the experience of Mixtec farmers indicates that propagation is the main problem in cuatomate production. Although sexual propagation is commonly successful and relatively simple, 50 to 60% of germinated plants are male (Medina-Galicia *et al*., 2011), as a result of the heterostyly phenomenon. Some plants produce fertile and long-styled flowers, while others have short-styled flowers that cannot be fertilized. Another method of propagation is by bud; however, this method is expensive, since a specialized laboratory is required. Therefore, vegetative propagation is an alternative to produce fertile plants: uniform plants with similar characteristics to the mother plant are obtained using this method (Hartmann *et al*., 2014). Vegetative propagation consists of separating a part of the mother plant (usually budwoods); this part is then placed under certain conditions and is induced to form roots and shoots, thus obtaining a new plant (Contreras G. and Almeida Puentes, 2006; Hartmann *et al*., 2014). The application of auxins to species that face difficulties rooting increases the possibility of obtaining plants by vegetative propagation and, in some species, it is essential for roots to form (Hartmann *et al*., 2014). Auxins are hormones that regulate plant growth; among other effects, they influence growth, cell division, and root formation. Therefore, they are frequently and increasingly applied in agriculture (Burgos *et al*., 2009; Garay-Arroyo *et al*., 2014). The most important natural auxin is indole-3-acetic acid (IAA) (Martínez *et al*., 2016). Therefore, the objective of this study was to establish the best concentration of indole-3-acetic acid and origin of cuatomate (*Solanum glacescens* Zucc.) budwoods for its vegetative propagation by cutting, as well as to describe the phenological stages —taking as parameters the time of budwood sprouts, and the time when the first bud, leaf, flower, and fruit appear—, in order to obtain fertile plants and contribute to their domestication process in the Low Mixtec region of Puebla.

MATERIALS AND METHODS

Location of the Experiment

The study was performed in a nursery covered with shade mesh, at the experimental field of the Instituto Superior de Acatlán de Osorio, in the municipality of Acatlán de Osorio (18° 11.9' N and 98° 3' W, at an altitude of 1180 m), Puebla, Mexico. The municipality of Acatlán de Osorio has an area of 483.48 km^2. Its climate transitions from dry —in the Lower Mixtec region of Puebla— to the warm —in the Valley region. Semi-warm subhumid climate with rains in summer has been identified, in the mountainous areas of the North and small areas in the Southeast-Southwest. The area between the mountainous areas and the lower parts of the municipality has a warm sub-humid climate with rains in summer. The latter area also has a very hot semi-dry climate.

Experimental Design

A completely randomized design with a 2^2 factorial design was used to estimate the effect of two concentrations of indole-3-acetic acid (1000 and 10000 ppm) and budwood type (secondary and tertiary) on the number of leaves and sprouts of the plant. The experimental and sampling unit was made up of one budwood. Twenty repetitions were carried out, obtaining a total of 80 experimental units.

Vegetative propagation by cutting of cuatomate

To propagate the cuatomate, budwoods taken from secondary and tertiary branches were used. The said branches were collected in September 2013, in the community of La Huerta, located in the municipality of Acatlán de Osorio. Forty 30-cm long budwoods from each type of branch (secondary and tertiary) were cut. A cross cut was made at one end of each budwood (the part that will give rise to the canopy), while a diagonal (wedge-shaped) cut was made at the other end (the part from which the root will grow). In the transversal cut, commercial candelilla wax was applied to prevent attacks by pathogens; the diagonal cut was moistened and subsequently fully immersed in the IAA hormone, using the concentrations mentioned in this study.

Phenology

For the description of the phenological stages, the first shoot of the first bud of 80 plants was registered, as well as the first leaf and flower. The number of leaves and sprouts were considered as response variables.

Fruit Evaluation

Fruit development was evaluated recording the diameter of the fruits of the biggest and the smallest plants, using a Mitutoyo 530-104 digital vernier caliper (standard and millimeter), with a range of 0-150 mm.

Statistical Analysis

Using the general linear models (GLM) procedure, an analysis of variance was carried out to determine the effect of IAA concentrations and budwood type on the number of leaves and the sprouts of the *cuatomate* plant. For each of the effects, the comparison of means was performed using the least significant difference method. The SAS (Statistical Analysis System, version 9.0) statistical package was used to perform all the analyses. An alpha value ≤0.05 was considered to be a significant effect. The following model was used to determine the effect of the treatments on the response variables (number of leaves and sprouts):

$$Y_{ij}=\mu+I_j+V_k+\varepsilon_{ij}$$

Where: Y_{ij}=Response of the observations obtained, expressed as number of leaves and sprouts in the i-th plant, as a result of the *j*-th concentration of indole-3-acetic acid and *k*-th budwood type. V_k=treatment. μ=World mean population. I_j=Effect of the *j*-th treatment. ε_{ij}=Experimental error of the observations obtained, expressed as number of leaves and sprouts in the *i*-th plant, as a result of *j*-th treatment.

RESULTS AND DISCUSSION

Estimation of the effect of the IAA concentration over the number of leaves and plant sprouts

The results of the statistical analysis showed that the IAA concentration, the budwood type, and their interaction significantly affect ($p\leq0.01$) the number of leaves and sprouts of the cuatomate. IAA concentrations had highly significant differences ($p\leq0.01$): 1000-ppm IAA budwoods had significantly more leaves and sprouts than 10000-ppm IAA budwoods (Figure 1).

Secondary and tertiary branches had highly significant differences ($p\leq0.01$) with regard to the number of leaves and sprouts: tertiary branches had a higher number of leaves and sprouts than secondary branches (Figure 2).

The analysis of the interaction of the IAA concentration with the budwood type showed a significant difference ($p\leq0.05$) in the number of leaves and sprouts; the TER1000

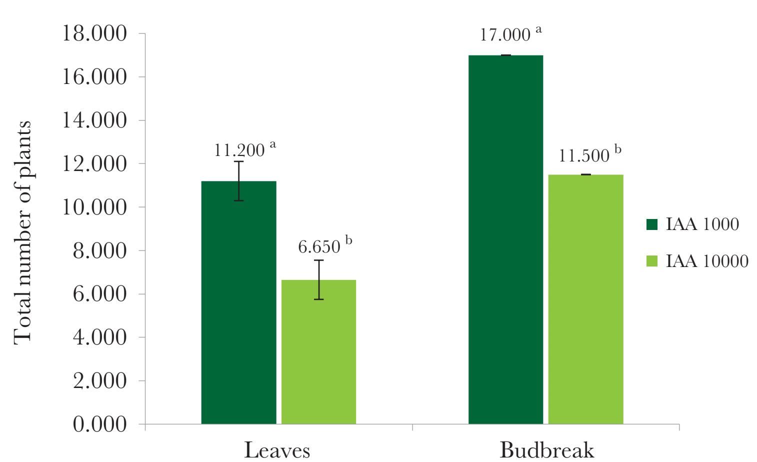

Figure 1. Number of leaves and budbreak due to IAA concentration. Means with different letters are statistically different ($p<0.01$).

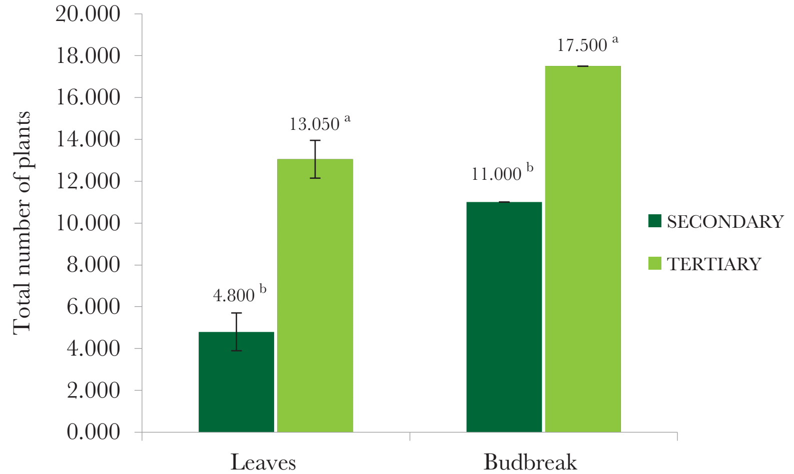

Figure 2. Number of leaves and budbreak due to secondary and tertiary rods. Means with different letters are statistically different ($p<0.01$).

(tertiary branch+1000 ppm of IAA) treatment showed more leaves and sprouts than other treatments (Figure 3).

Studies about the cuatomate plant have mainly been focused on its importance as an endemic plant that grows wild in the Mixtec region, where it is framed as an important food source in rural areas (Gutiérrez-Rangel *et al.*, 2011; Martínez-Pérez *et al.*, 2012). According to Gutiérrez-Rangel *et al.*, (2011), Martínez (2004) studied the cuatomate adaptation to an intensive production system and determined that the use of 50% half shade is suitable for the development of this species; however, Martinez (2004) also established that the application of different nitrogen, phosphorus, and potassium concentrations had no significant effect on the cuatomate production. There are not many studies about the vegetative propagation

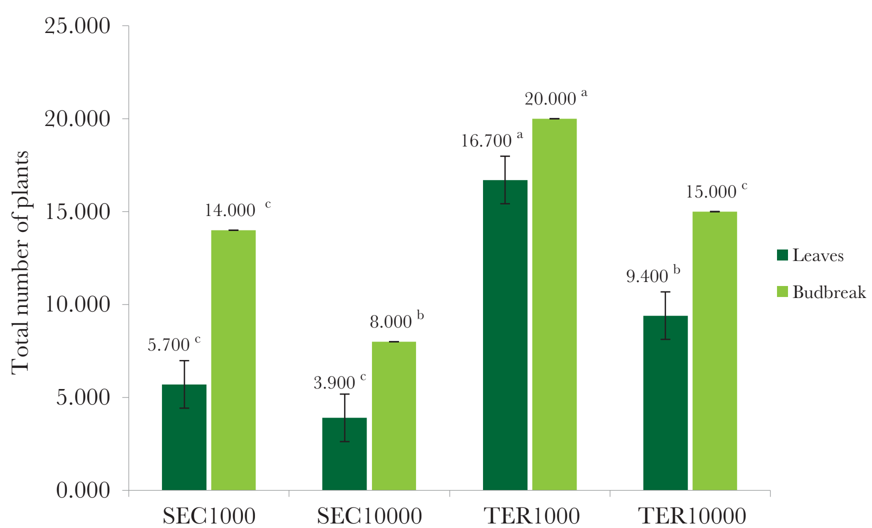

Figure 3. Number of leaves and budbreak due to the treatments. Means with different letters are statistically different ($p<0.01$). SEC1000: Secondary to 1000 ppm; SEC: Secondary to 10000 ppm; TER1000: Tertiary to 1000 ppm; TER: Tertiary to 10000 ppm.

of this species, although the use of auxins increases the possibility of obtaining plants by vegetative propagation in species that have difficulties establishing roots (Hartmann *et al.*, 2014). IAA is the most important natural auxin and its application in some crops, such as zucchini (*Cucurbita pepo* L.), is related to the sprouts and growth of the fruit (Li *et al.*, 2005). The same behavior was found in melon crops: significant differences in number of fruits/plants was observed, when 50-, 100-, 150-, and 200-mg L^{-1} doses of IAA were applied (Montaño-Mata and Méndez-Natera, 2009).

Phenological Evaluation

Phenological stages refer to the periodic rhythm or biological stages of a plant such as flowering, shoots, and ripening of the fruit. These phenomena are related to the local climate (Pérez-Hernández *et al.*, 2018; Solórzano-Vega, 2007). The first bud was observed 30 days after IAA inoculation, while the first leaf and flower appeared 40 and 180 days after inoculation, respectively (Table 1 and Figure 4).

Fruit Evaluation

First bud, leaf, and flower are indicators of fruit generation. The fruit has a gradual development; industrialization requires ripe fruits (Figure 5). The average growth was 0.02 mm in diameter per day in a period of 23 days (Table 2); in average, the plant started to bear fruits 8 months after the sprouts; the first harvest was obtained after a year. The average diameter at the ripening stage was 3.8 cm. The average weights obtained during the experiment were 30-38 g.

According to Medina (2011), the main physical and physiological aspects of the cuatomate crop —such as its reproduction, handling, and conservation— are not well known; however, in other studies, Medina also shows that its commercial significance lies in the following aspects: cuatomate is only consumed by 51% of producers; 41% both consume it and sell it; while the remaining 8% considered it as a hugely relevant part of their culture, as a traditional element of the family diet over generations.

CONCLUSIONS

The highest number of leaves (11.2) and sprouts (17) were obtained from tertiary budwoods inoculated with 1000 ppm of IAA. The first bud was observed 30 days after IAA inoculation, while the first leaf and flower appeared 40 and 180 days after inoculation, respectively. The plant began to bear fruits after an average of 8 months; the first harvest

Table 1. Phenological stages of the cuatomate during the experiment.

Activity	Date	Days
Budwood collection	09/26/2013	01
Hormone application and bag placement	09/26/2013	01
First bud	10/26/2013	30
First leaf	11/05/2013	40
First flower	03/27/2014	180

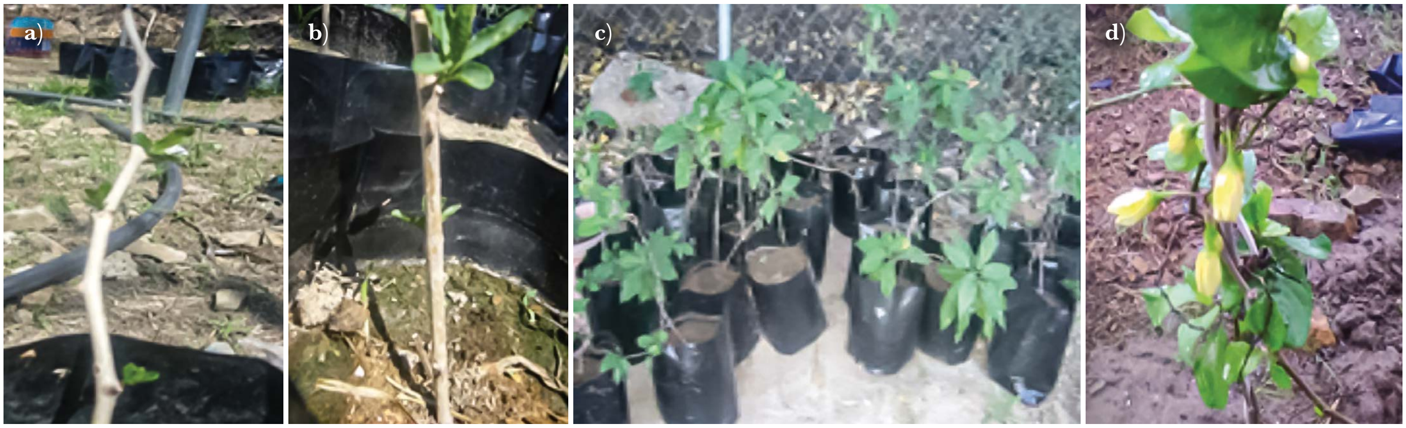

Figure 4. Phenological stages of cuatomate during the experiment: a) Shoot of the first leaf, b) Formation of leaves, c) Formation of leaves and small branches, d) Flowering of the cuatomate.

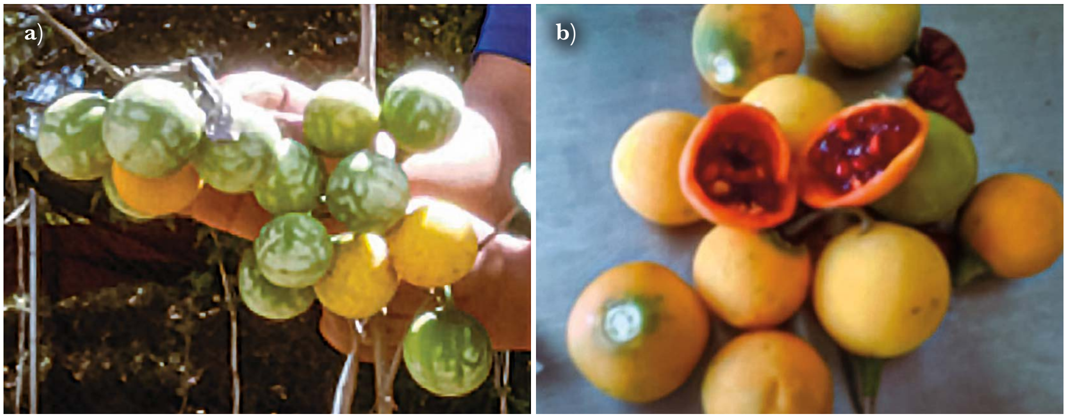

Figure 5. Cuatomate in its different ripening stages: a) Cluster of physiologically mature cuatomate, b) Ripe cuatomate ready to prepare salsa roja.

Table 2. Development of the cuatomate fruit during the experiment.

Date	06/25/2014	06/26/2014	06/30/2014	07/04/2014	07/08/2014	07/14/2014	07/18/2014
Plant 1	0.5 cm	0.52 cm	0.55 cm	0.55 cm	0.64 cm	0.7 cm	0.9 cm
Plant 2	3.37 cm	3.4 cm	3.56 cm	3.59 cm	3.60 cm	3. 61 cm	3.63 cm

was obtained after one year. The use of IAA in the vegetative propagation of the cuatomate would be an alternative to increase its production, as a result of its widespread acceptance in the regional gastronomic industry and its commercialization in the United States by Mixtec emigrants. It would also be a sustainable activity for the Mixtec region of Puebla.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

An *in vitro* and *in situ* evaluation of a diet for cattle added with organic oils

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ABSTRACT

Objective: To perform *in vitro* and *in situ* evaluation of a diet for dairy cattle at different rates of fish oil and soybean oil.

Designmethodology/approach: Four treatments with different rates of fish oil (FO) and soybean oil (SO) were evaluated (Control: no added oil; diet 1: 2% FO; diet 2: 2% FO and 1.5% SO; diet 3: 2% FO and 3% SO). *In vitro* digestibility and *in situ* degradability were evaluated. Ammonia nitrogen, lactic acid, volatile fatty acids (VFAs), and microbial protein were determined. For the *in situ* evaluation, a protein degradability kinetic was carried out. The means were compared using a Tukey test at a 5% confidence level.

Results: The proposed diets increased gas production in *in vitro* kinetics, as the addition of oils increased ($p<0.001$) and the kinetic latency time decreased ($p<0.001$). All diets decreased the production of short-chain fatty acids ($p<0.001$). The production of ammonia nitrogen and lactic acid did not differ compared to the control ($p<0.05$). Diet 3 had a higher production of propionic acid in comparison to diet 1 and 2. In the *in situ* kinetic, the "*kd*" rate increased as more oils were added.

Study limitations/implications: Although all treatments increased the production (milliliters) of CH_4 and CO_2, the gas production had a proportional increase, as a result of a better use of the diets.

Findings/conclusions: The addition of oils produced changes in the fermentation patterns and in the degradation of the protein at the ruminal level, increasing bypass protein. This offers an opportunity to improve performance in certain production situations.

Keywords: *in vitro* digestibility, *in situ* degradability, gas production.

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INTRODUCTION

Conjugated linoleic acid (CLA) —a fatty acid found naturally in the dairy and meat products of ruminants— has been identified as a potential anti-obesogenic, anti-cancerogenic, and enhancer of the immune and inflammatory response (Den Hartigh, 2018; Whigham, Watras, and Schoeller, 2018). CLA is an 18-carbon PUFA formed as an intermediate, during the biohydrogenation (BH) of linoleic acid (C18:2 *cis*-9, *cis*-12, LA) and alpha-linolenic acid (C18:3 *cis*-9, *cis*-12, *cis*-15, ALA), and their transformation into

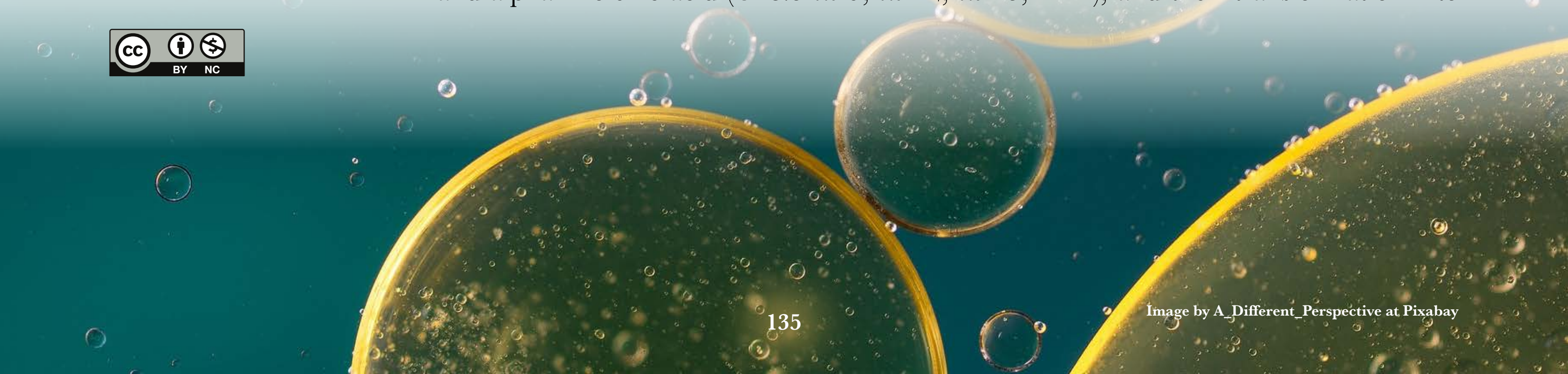

Image by A_Different_Perspective at Pixabay stearic acid (C18:0, SA), or by the endogenous conversion of *trans*-vaccenic acid (C18:1 t-11, TVA) by the action of the Δ9-desaturase enzyme in the mammary gland (Khanal and Olson, 2004). LA and ALA are found in high proportions in the lipids of fodder and in some supplements, such as oils. Castillo *et al.* (2013) point out that supplementing diets with LA- and ALA-rich soybean oil reduces short-chain fatty acids and increases long-chain unsaturated fatty acids (UFA) —especially TVA and CLA. For its part, fish oil contains eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA); adding these acids to ruminant diets increases CLA concentration in milk and meat (Wąsowska *et al.*, 2006). This has an impact on the BH process of the UFA, increasing the contribution of TVA —which serves as a substrate for the endogenous formation of CLA (Shingfield *et al.*, 2006).

Furthermore, ruminants make a significant contribution to the total greenhouse gas (GHG) emissions of the agricultural sector (Sejian and K. Naqvi, 2012). One way to decrease methanogenesis in ruminants is to include PUFAs in the diet, since UFAs serve as electron acceptors during the biohydrogenation process, causing a depression in methane production (Broucek, 2018). Methane represents a significant loss of dietary energy: a 2-12% loss of gross energy intake (Johnson and Johnson, 1995). Reducing enteric methane production could also increase feed efficiency.

Taking into account that both the production of GHG and the fatty acids accumulated in the milk and meat of the ruminant take place at the metabolic level, determining the effects of the addition of organic oils in its diet is an important step. Therefore, the objective of the present study was to evaluate the effects of adding FO and SO on the parameters of ruminal fermentation and the *in vitro* and *in situ* degradability kinetics. This evaluation allowed us to find out the metabolic differences between the different oil levels.

MATERIALS AND METHODS

The evaluated diets contained a balanced feed mixture (6% soybean husk, 16% distillers' dried grains, 6% sugarcane molasses, 20% rolled corn, 17.5% ground corn, 3.5% mineral mixture, 4% cottonseed, 5.8% soybean paste, 11.2% ground sorghum, 4% cottonseed meal, and 6% wheat bran), silage, and alfalfa (30, 29, and 41%, respectively) added with different ratios of SO and FO (Control: without added oil; diet 1: 2% FO; diet 2: 2% FO and 1.5% SO; diet 3: 2% FO and 3% SO). For the gas production technique proposed by Menke *et al.* (1979), the components of the diets were dried in an oven at 55 °C for 48 h, ground, and sieved (1mm mesh) separately. One gram of dry matter (DM) from each diet (with their respective oils) was added to 250-mL fermentation flasks with 120 mL of a 2:1 mixture of ruminal fluid and artificial saliva (Macromineral solution: 1 L distilled H_2O, 5.7 g Na_2HPO_4, 6.2 g KH_2HPO_4, 0.6 g $MgSO_47H_2O$; micromineral solution: 100 mL distilled H_2O, 13.2 g $CaCL_2 \bullet H_2O$, 10 g $MnCl_2 \bullet 4H_2O$, 1 g $CoCl_2 \bullet 6H_2O$, and 8 g $FeCl_3 \bullet 6H_2O$; buffer solution: 1 L distilled H_2O and 39 g $NaHCO_3$; reducing solution: 100 mL distilled H_2O, 4 mL 1N NaOH and 625 mg $Na_2S \bullet 9H_2O$; and resazurin solution: 100 mL distilled H_2O and 100 mg resazurin); CO_2 was used to establish an anaerobic environment; the fermentation flasks were incubated in an ANKOM Daisy^{II} equipment for 96 h. The accumulated gas pressures were measured using an automated measuring equipment (ANKOM RF Gas production System) equipped with a pressure transducer connected to each fermentation flask. Measurements were taken at 0, 0.5, 1, 2, 3, 6, 12, 24, 36, 48, 72, and 96 h after inoculation. The gas production profiles were adjusted to the Gompertz equation (Tjørve and Tjørve, 2017) (Equation 1).

$$GP = Gmax\ e^{-Ae^{-kt}} \tag{1}$$

Where GP=gas production, $Gmax$=maximum gas production, A=adaptation phase, and k=gas production rate.

Gas samples were taken from the upper section of the fermentation flasks at 24 h, to measure the production of CO_2 and CH_4, using a Biogas 5000 equipment. At the end of the fermentations (96 h), the residues of fermentation were centrifuged at 2,500 rpm for 5 min, then they were filtered, and used to analyze VFAs, ammonia nitrogen, and lactic acid. The VFAs were analyzed in a 6890N gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a flame ionization detector and an HP-Innowax polyethylene glycol capillary column (30 m×0.32 mm×0.15 μm, J&W Scientifics). The oven was set up at 80 °C; after 1 min, the temperature was raised to 120 °C, with a 20 °C/min increase, until it reached 205 °C; afterwards, the temperature was increased 10 °C/min (for 2 minutes). Nitrogen was used as carrier gas at a constant flow rate of 40 ml/min, injecting 1 μL of sample.

The ruminal environment was evaluated through the analysis of ammonia nitrogen, lactic acid, *in vitro* digestibility of dry matter (*IVDDM*), and microbial protein. The ammonia nitrogen in the samples (Galyean, 2010) was subject to a spectrometry analysis at a wavelength of 630 nm in a Genesys 10S VIS Thermo Scientific spectrometer; the lactic acid in the samples (Borshchevskaya *et al.*, 2016) was analyzed using the same equipment at 390 nm with 0.2% $FeCl_3$. The *IVDDM* was evaluated using nylon bags F57 (ANKOM)s with 0.5 g of each diet in 1.6 L of a mixture of solution A:solution B (5:1) (Solution A: 10 g/L KH_2PO_4, 0.5 g/L $MgSO_4$, 0.5 g/L NaCl, 0.1 g/L $CaCl_2 \cdot 2H_2O$, and 0.5 g/L urea; Solution B: 15 g/L Na_2CO_3 and 1 g/L $Na_2S \cdot 9H_2O$) and 400 mL of ruminal fluid; 5-L glass incubation jars were used in an ANKOM Daisy^{II} incubator at 39 °C for 48 h; CO_2 was used to establish an anaerobic environment. At the end of the incubation period, the nylon bags F57 (ANKOM) were dried in an oven at 55 °C for 48 h and the *IVDDM* was determined by weight difference. The *IVDDM* value of each diet and its gas production at 48 h were used to calculate the microbial protein using Equation 2, according to Blümmel, Steingaβ, and Becker (1997).

$$MP\left(\frac{mg}{g\ \mathrm{de}\ MS}\right) = IVDDM - \left(GP_{48} * 2.2\right) \tag{2}$$

Where: MP=microbial protein, $IVDDM$=*in vitro* digestibility of dry matter, and GP_{48}=gas production at 48 hours.

The *in situ* evaluation was carried out in three "Angus" cattle with ruminal fistula, using the nylon bag technique (Mehrez & Ørskov, 1977): 20×10 cm bags with an average pore of 50 microns were used. The bags were previously dried at 60 °C for 24 h; 10 g of DM of each diet (with their respective oils) were placed in each one; and two repetitions of each time were made (0, 3, 6, 12, 18, 24, 36, 48, 72, and 96 h). The bags were placed in the ventral part of the rumen and were introduced in the reverse order of their incubation time; all of them were removed together. The zero-hour bags were introduced and immediately removed, once they were moistened with ruminal fluid (Nocek & Russell, 1988). The bags were thoroughly washed with running water and then dried in an oven at 55 °C for 48 h. Crude protein (CP) was determined from the residues of each bag with the Kjeldahl method, using the N×6.25 factor (AOAC 991.20). *In situ* degradation parameters were estimated with the modifications made by McDonalds (1981) to the model proposed by Orskov and Mcdonald (1979) (Equation 3).

$$d = a + b\left(1 - e^{-kd*t}\right) \quad (3)$$

Where: d=degradability at t time, t=incubation time, a=soluble or rapidly degradable fraction, b=insoluble but potentially degradable fraction (%), and kd=degradation constant of "b".

The non-digestible fraction ("C") and potentially digestible fraction ("PD") were calculated using Equation 4, and the effective degradability ("ED") was calculated using Equation 5.

$$C = 100 - (a + b) \quad (4)$$

Where: C=non-digestible fraction (%), PD=potentially digestible fraction ($a+b$).

$$ED = a + \left[(b * kd) / (kd + kp)\right] \quad (5)$$

Where: ED=effective degradability, kp=5%/h constant ruminal passage rate, and kd=degradation constant of "b".

A completely randomized experimental design was used; a mean comparison Tukey test was performed. We considered that there was a significant difference when $p<0.05$. A statistical analysis was performed with the SigmaPlot [12.0] statistical software.

RESULTS AND DISCUSSION

In the parameters obtained for the Gompertz equation (Table 1), the maximum gas production ("*Gmax*") increased ($p<0.001$) as more oil was added and the "A" lag phase decreased ($p<0.001$). In diet 3, the "k" gas production rate increased ($p<0.05$) compared to control. The increase in "*Gmax*" indicates a greater fermentation of organic matter (Blümmel and Ørskov, 1993). This increase differs from the results of Toral *et al.* (2009)

in sheep, who used a mixture of FO with sunflower oil (1 and 2%), observing a slight decrease in the accumulated gas production, without changing the gas production rate. These differences may be the result of the type and quantity of oils, as well as of the different concentrate rates used in each study (Wachira *et al*., 2000; Ueda *et al*., 2003; Shingfield *et al*., 2008). Beuvink and Spoelstra (1992) point out that the total amount of gas produced depends on the composition of the final fermentation products. Starch fermentation produces more gas than glucose or cellulose fermentation. Therefore, the increase in "*Gmax*" obtained in the present study can be attributed to an increase in the population of amylolytic bacteria, as a consequence of the toxic effect that oils have on cellulolytic bacteria and protozoa (Harfoot and Zealand, 1997; Yang *et al*., 2009; Palmquist and Jenkins, 2017; Roy *et al*., 2017; Lima *et al*., 2019). The decrease in the number of cellulolytic bacteria could also explain the decrease in total volatile fatty acids (VFA) (Table 4), since cellulose fermentation produces the highest quantity of VFAs (Beuvink and Spoelstra, 1992).

Within the parameters of ruminal fermentation (Table 2), the *IVDDM* decreased in diet 2 (63.9%) compared to control and diet 1 (68.2 and 67%, respectively; $p<0.05$). The ammonia nitrogen and lactic acid concentration is not affected by the addition of oils compared to the control diet. Microbial protein synthesis decreased as more oils were added ($p<0.001$). The decrease in *IVDDM* observed in diet 2 differs from the results obtained by Roy *et al*. (2017), who did not observe differences in *IVDDM* when they used different oils (0, 3, and 4%). In contrast, El-Sherbiny *et al*. (2016) observed a decrease in *IVDDM* with FO and SO mixtures at 5 and 7% of total addition.

Table 3 shows an increase ($p<0.001$) in the production of CO_2 and CH_4, related to higher gas production (*Gmax*). On one hand, the ratio is not significantly affected, indicating that diets do not change the rate in which it is produced. No significant differences were

Table 1. Effect of the addition of organic oils on the parameters of the Gompertz equation.

	Control	DIET 1	DIET 2	DIET 3	EE*	p-value
Gmax (mL)	62.1 ± 3.38^{c}	96.6 ± 4.01^{b}	138.7 ± 12.09^{a}	140.4 ± 10.46^{a}	18.75	<0.001
A (h)	15.5 ± 2.21^{a}	7.2 ± 1.09^{b}	4.5 ± 0.59^{bc}	3.7 ± 0.21^{bc}	2.69	<0.001
k (h^{-1})	0.06 ± 0.004^{b}	0.14 ± 0.029^{ab}	0.24 ± 0.117^{ab}	0.29 ± 0.075^{a}	0.05	<0.05

a, b, c, d: the same letters indicate that there is no statistically significant difference, EE*: Standard error.

Table 2. Effect of the addition of organic oils on the parameters of ruminal fermentation.

	Control	DIET 1	DIET 2	DIET 3	EE*	p-value
DIVMS: (%)	68.2 ± 1.20^{a}	67.0 ± 2.21^{a}	63.9 ± 2.42^{b}	65.9 ± 1.10^{ab}	0.91	<0.05
Ammonia nitrogen (mg/dL)	13.0 ± 1.48^{ab}	10.8 ± 1.72^{b}	13.8 ± 0.39^{a}	12.8 ± 0.04^{ab}	0.63	<0.05
Lactic acid (g/L)	2.6 ± 0.38^{ab}	3.0 ± 0.12^{a}	2.1 ± 0.18^{b}	2.2 ± 0.28^{ab}	0.20	<0.05
Calculated microbian protein (mg/g DM)	564.6 ± 16.11^{a}	462.9 ± 17.1^{b}	364.6 ± 37.39^{c}	381.0 ± 14.57^{c}	45.80	<0.001

a, b, c, d: the same letters indicate that there is no statistically significant difference, EE*: Standard error. *In vitro* digestibility of dry matter (*IVDDM*).

Table 3. Effect of the addition of organic oils on the production of methane and carbon dioxide.

	Control	DIET 1	DIET 2	DIET 3	EE*	p-value
CO_2 (ml)	33.1±1.14[c]	64.4±0.02[b]	58.7±5.05[b]	83.0±7.94[a]	10.30	<0.001
CH_4 (ml)	5.1±0.08[c]	10.3±0.14[b]	9.5±0.31[b]	12.7±0.52[a]	1.58	<0.001
CO_2 (%)	71.7±0.25	76.9±0.3	75.6±3.7	73.1±3.32	1.17	nssd
CH_4 (%)	11.2±0.60[b]	12.3±0.15[a]	12.2±0.05[a]	11.2±0.25[b]	0.30	<0.05
CH_4/CO_2	0.15±0.007	0.16±0.002	0.16±0.008	0.15±0.008	0.002	nssd

a, b, c, d: the same letters indicate that there is no statistically significant difference, nssd: no statistically significant difference, EE*: Standard error.

found in the percentage of that was produced. On the other hand, the increase of the propionic acid production (Table 4) in diet 3 and the control compared to diets 1 and 2 (23.8 and 23.1%: 16.7 and 18.3%, respectively; $p<0.05$), decreases the percentage of (11.2 and 11.2%: 12.3 and 12.2%, respectively; $p<0.05$), as well as the A/P ratio (1.8 and 1.9: 2.8 and 2.7, respectively; $p<0.05$). The production of TVFAs decreased with the addition of oils ($p<0.001$); consequently, there was a greater decrease in diet 3 (10.1 mM) than in control (17.5 mM). These results match the findings of several authors (Broudiscou and Lassalas, 1991; Zhang *et al.*, 2008; Martin *et al.*, 2009; Vargas *et al.*, 2020); as propionic fermentation increases, the hydrogen uptake increases and methane production decreases (Boadi *et al.*, 2004).

In the case of *in situ* protein degradability (Table 5), diet 3 presents highly significant effects ($p<0.001$) in the "*C*" non-digestible fraction —*i.e.*, a greater increase with respect to control (48.9 and 41.0%, respectively). Likewise, its potentially digestible fraction (*PD*) shows a greater decrease (51.0 and 58.9%). A similar effect is observed in diet 1 and 2. The *ED* in diets 2 and 3 is greater than in control (31.6 and 30.6: 25.5%, respectively; $p<0.05$); a similar effect is observed with "*kd*" (0.045 and 0.061: 0.025 h^{-1}, respectively; $p<0.001$). The "*a*" fraction of the diets did not present a significant difference ($p>0.05$) compared to control. The decrease in the *PD* fraction and increase in *C* is consistent with a study carried out by Ferreira *et al.* (2016) in lambs, in which the digestibility of CP in the total digestive tract decreased when a mixture of FO and SO was supplemented: the higher the SO concentration, the lower the apparent digestibility. This decrease in protein degradability is related to the decrease in microbial protein synthesis (Table 2), which could be the result of an inhibition in microbial growth caused by the PUFAs present in FO and SO (Maia *et*

Table 4. Effect of the addition of organic oils on the production of volatile fatty acids (VFAs).

	Control	DIET 1	DIET 2	DIET 3	EE*	p-value
Acetic acid (%)	44.2±6.92	46.5±2.63	48.6±2.73	44.7±1.78	0.99	nssd
Propionic acid (%)	23.1±0.94[a]	16.7±1.41[b]	17.6±1.15[b]	23.8±1.78[a]	1.83	<0.05
Butiric acid (%)	27.8±5.97	31.9±1.22	28.8±2.65	26.6±3.56	1.13	nssd
TVFA (mM)	17.5±1.11[a]	11.8±1.12[bc]	13.1±0.99[b]	10.1±0.40[c]	1.58	<0.001
A/P ratio	1.9±0.37[b]	2.8±0.39[a]	2.7±0.16[a]	1.8±0.06[b]	0.26	<0.05

a, b, c, d: the same letters indicate that there is no statistically significant difference, nssd: no statistically significant difference, EE*: Standard error.
TVFA: total volatile fatty acids.

Table 5. Effect of the addition of organic oils on the *in situ* ruminal degradation parameters and effective protein degradability of each diet.

	Control	DIET 1	DIET 2	DIET 3	EE*	p-value
a (%)	8.3±2.49[ab]	13.4±0.33[a]	7.6±1.75[ab]	5.7±2.78[b]	1.64	<0.05
b (%)	50.5±4.22	43.2±0.15	50.1±1.54	45.3±3.64	6.73	nssd
kd (h^{-1})	0.025±0.0001[c]	0.027±0.005[c]	0.045±0.000[b]	0.061±0.0032[a]	0.008	<0.001
Non-digestible fraction "*C*" (%)	41.0±1.72[c]	43.2±0.48[b]	42.1±0.21[bc]	48.9±0.86[a]	1.75	<0.001
Potentially digestible fraction "*DP*" (%)	58.9±1.72[a]	56.7±0.48[b]	57.8±0.21[ab]	51.0±0.86[c]	1.75	<0.001
Effective degradability "*DE*" (%); para *kp*=5%/ h	25.5±1.12[b]	28.8±1.62[ab]	31.6±1.17[a]	30.6±1.36[a]	1.34	<0.05

[a, b, c, d]: the same letters indicate that there is no statistically significant difference, nssd: no statistically significant difference, EE*: Standard error, C=non-digestible fraction ($C=100-(a+b)$), *PD*= potentially digestible fraction ($PD=a+b$), *ED*: effective degradability for kp=5%/hour ($ED=a+[(b*kd)/(kd+kp)]$).

al., 2010; Ferreira *et al.*, 2016). These findings match the tests carried out by El-Sherbiny *et al.* (2016), who recorded a reduction in the total count of bacteria as a mixture of SO and FO increased to 3, 5, and 7% of total addition.

CONCLUSIONS

The addition of oils produced changes in the fermentation patterns that suggest a modification in the microbial populations. Protein degradation is also modified, increasing bypass protein. This offers an opportunity to improve performance in certain production situations.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Agronomic practices and bio-stimulants for persian lime (*Citrus latifolia* Tan.) production in Mexico

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ABSTRACT

Objective: To evaluate biostimulants and cultural practices for floral induction in Persian lime (*Citrus × latifolia* Tanaka ex Q. Jiménez) to obtain production during the winter season, when the highest price in the market is offered with.

Design/methodology/approach: From July to September period, biostimulants and cultural practices were applied to six-year-old trees with different treatments as follows: 1. Pruning only, 2. Pruning + urea, 3. Pruning + urea + ringing, 4. Pruning + biofol® + ringing. Additionally, during September, two more treatments were included: 5. Pruning + calcium prohexadione, and 6. Pruning + Citomax®. The design used was randomized blocks with four repetitions. The experimental unit was one lime tree. Flowering, yield, physical-chemical quality and cost-benefit ratio were evaluated.

Results: The outstanding effect of pruning and the effect of nitrogen were confirmed with the foliar application of granulated urea (6.0 kg ha^{-1}) during the period from July to September, obtaining an average yield of 23 t ha^{-1} during the harvest from December to March, with a B/C 2.5 ratio. The quality of the fruit was kept within the NMX-FF-077-1996 Mexican standard. September applied Pruning + Citomax® (cytokinins) showed a yield of 30 t ha^{-1}, with a B/C 3.1 ratio.

Findings/conclusions: Pruning + nitrogen, and pruning + cytokinins induce flowering and produce Persian lime with the best winter yields.

Keywords: quality fruit, pruning, phytohormones, productivity.

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INTRODUCTION

In Mexico, Persian lime (*Citrus × latifolia* Tanaka ex Q. Jimenez) is produced between May and September, when supply is greater and prices are low, making production unprofitable; winter production has better prices due to their scarce supply. Through

pruning practices, banding and application of biostimulants from July to September, high yields are obtained during winter, with better market prices, increasing the productivity and income competitiveness of Mexican lime producers (Ariza *et al.*, 2004; Ariza *et al.*, 2015), so these practices were applied to other citrus fruits.

In Mexico, Persian lime is grown in over 72 thousand ha, with an average annual yield of 13.5 t ha^{-1}, the state of Morelos being the fourteenth in surface area with 349 ha and ninth in average yield with 11.4 t ha^{-1} of limes (SIAP, 2019). Technologies relating biostimulants application (Ariza *et al.*, 2015) or cultural practices on plants such as banding, total fruit harvest, water stress and pruning (Ariza *et al.*, 2004) or combined have been recently evaluated (Ambriz *et al.*, 2013; Ambriz *et al.*, 2013 a; Ambriz *et al.*, 2018). There is a necessity to explore new technologies to avoid plant wear in a sustainable and maintained way, to contribute to the productivity and competitiveness of lime plantations. By improving the technological level of orchard care and researching the application timing of agronomic practices and biostimulants for Persian lime production and competitiveness during winter. Subsequently making these results accessible to producers and with it offer an alternative to improve their production and income.

The objective of this research was to evaluate and generate technologies for flowering and to increase the production and profitability of Persian lime, along with the evaluation of the effects of agronomic practices and biostimulants on fruit quality.

MATERIALS AND METHODS

Trials were established at Ahuahuetzingo, Puente de Ixtla, state of Morelos, Mexico, to evaluate biostimulants and cultural practices on six-year-old Persian lime trees, with a density of 357 trees per ha, managed following directions by Alia *et al.* (2011).

Five treatments were established throughout July, August and September 2018: a) pruning, b) pruning + 46% granular urea (6.0 kg ha^{-1} foliar application), c) pruning + 46% granular urea (6.0 kg ha^{-1} foliar application) + branch banding, d) pruning + biofol® (3 L ha^{-1} foliar application). Two additional treatments were added during September: e) pruning + calcium prohexadione (3.0 kg ha^{-1} foliar application), f) pruning + cytokinins (Citomaxx® at 3 L ha^{-1} foliar application). The employed assessment had a randomized complete block design with four replications, one tree as experimental unit.

Variables were obtained from four branches to quantify the number of flowers and marble-like fruits (<1.0 cm in diameter); from each tree, the number of fruits per individual, the fruit weight per tree was determined with a clock scale and expressed in kg (Ohaus® brand), and the yield was calculated as kg ha^{-1}. From 10 fruit samples, fruit quality was determined as fruit mass, polar and equatorial diameters were assessed with a Vernier (Mitutoyo® brand), total soluble solids determined with a refractometer (Hanna® brand, model HI96801) and expressed as °Brix and juice content in mm (Alia *et al.*, 2009). Profitability was also determined for competitiveness with the production costs and production value in Mexican pesos through by the benefit/cost ratio (B/C).

Five fruit harvests were carried out on the following dates: November 27-30, January 15-17, February 9-11, March 5-6 and March 25-26, to obtain the total sum of the sum of each of the evaluated treatments on the plants.

With the results of the three experiments and analysis of variance (ANOVA) was performed according to the experimental design and the DMS mean comparison tests in the SAS statistical software (Anonymous, 2015).

RESULTS AND DISCUSSION

During the trial conducted in July 2018, the number of flowers, number of fruits, weight of fruits per tree and total yield were significantly higher in the Persian lime trees in which pruning + urea or pruning + urea + banding was applied, compared to the rest of the treatments (Table 1). Pruning promoted new shoots formation, both vegetative and reproductive, which were part of the flowering and production (Ariza *et al.*, 2004); the effect of urea was to convert ammonium, reduce ethylene production and induce flowering (Lovat *et al.*, 1988). Similar results were reported by Ambriz *et al.* (2018); they concluded pruning, urea application and banding in September to promote flower budding and fruit setting in Persian limes for winter production. No significant differences attributed to the treatments were detected in the fruit weight variables, polar and equatorial diameter, juice percentage and total soluble solids content (Table 1).

During the August 2018 assessment, the largest number of tree flower was recorded in the pruning + urea treatment (Table 2); the highest fruit number, fruit weight and yield, were determined, on the trees with the pruning + urea + banding performance (Table 2). Trees which were only pruned showed the lowest values of yield components (Table 2).

In the September 2018 evaluation, the results indicate significant differences in the number of flowers, number of fruits, weight and kilograms per hectare yield (kg ha^{-1}); it is also observed that in the pruning + cytokinins treatment differences on the polar diameter quality variable existed, this did not occur for the rest of the evaluated treatments (Table 3 and Figure 1). There are no reports about the effect of cytokinins on flowering and fruit production in citrus plants, but there are in cv. Golden Delicious/MM111 apple, that indicate that cytokinins individually applied or combined with calcium

Table 1. July experiment results for flowering variables, yields and fruits quality using biostimulants and agronomic practices for floral induction and production of Persian lime during winter at the state of Morelos, Mexico. July 2018/2019 period.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	48 C	389 bc	43.7 bc	15.6 ab	132	73.4	59.7	46	9.5
Pruning + urea	101 a	572 a	60.9 a	21.7 a	136	74.3	60.4	44	9.4
Pruning + urea + ringed	83 ab	509 ab	56.9 ab	20.3 a	133	72.8	61.2	48	9.4
Pruning + Biofol® + ringed	65 bc	309 c	37.0 c	12.4 b	131	71.9	60.7	45	9.4
D.M.S.	32.8	126	17.2	6.2	22	4.9	3.7	5.2	0.9
C.V.	27.7	18.3	22.4	23.0	10.7	4.3	4.0	7.4	6.6
Significance	*	*	*	*	n.s.	n.s.	n.s	n.s.	n.s.

Means with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

Table 2. Results of the August assessment on flowering, yield and fruit quality variables when using biostimulants and agronomic practices for floral induction and production of Persian lime during winter at the state of Morelos. August period 2018/2019.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	61 ab	429 b	48.9 b	17.5 b	135	73.0	60.7	46	9.1
Pruning + urea	91 a	615 ab	72.2 ab	25.8 ab	136	73.4	61.3	46	9.0
Pruning + urea + ringed	56 b	789 a	84.4 a	30.1 a	139	74.4	62.1	47	9.3
Pruning + Biofol® + ringed	78 ab	476 b	55.6 ab	20.1 ab	136	74.0	61.0	45	9.6
D.M.S.	30.5	236	28.7	10.2	26.8	5.9	2.9	6.7	0.9
C.V.	27.7	26.6	28.5	28.5	12.7	5.1	3.1	9.6	6.6
Significance	*	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s

Averages with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

prohexadione (P-Ca) produced heavier fruits, increased the carotenoids and vitamin C concentration and increased the induction of flower buds (Ramírez *et al.*, 2017), calcium prohexadione stimulated flowering and production in cv. Red Delicius apples (Kiessling *et al.*, 2008). Palmer *et al.* (2015) also demonstrated the cytokinins potential to induce flower bud development in cv. Scifresh/M9 apple trees, concurring with this experiment. Corbesier *et al.* (2003) reported cytokinins accumulation in leaves, leaf exudates and apical meristems, all of which correlate with early events of the flowering transition in *Arabidopsis thaliana* (L.).

There were highly significant differences, mainly for yield (t ha^{-1}), due to the applied practices and biostimulants. If only pruning is performed, the average yield is 16.8 t ha^{-1}

Table 3. Results of flowering, yield and fruit quality variables during the September assessment using biostimulants and agronomic practices for floral induction for Persian lime production during winter at the state of Morelos, Mexico. September 2018/2019 period.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	45 ab	465 bc	48.9 bc	17.5 bc	127	70.1ab	60.2	48	9.3
Pruning + urea	47 ab	592 ab	63.3 b	22.6 b	138	72.6ab	60.5	47	9.2
Pruning + urea + ringed	45 ab	442 bc	51.8 bc	18.5 bc	143	72.7ab	61.9	47	9.3
Pruning + Biofol® + ringed	33 b	361 c	42.2 c	15.0 c	141	75.2a	60.9	46	9.4
Pruning + Prohexadione calcium	53 a	442 bc	49.5 bc	17.6 bc	140	71.9ab	61.5	44	9.3
Pruning + cytokinins	50 a	694 a	85.0 a	30.3 a	134	66.6b	60.7	45	9.6
D.M.S.	17.7	192.7	19.9	7.13	22.2	4.5	2.7	7.3	1.0
C.V.	26.8	25.9	23.6	23.7	10.9	4.0	2.9	10.7	7.3
Significance	*	**	**	*	n.s.	*.	n.s.	n.s.	n.s.

Averages with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

Figure 1. Appareance of Persa lemon fruits by pruning branches treatment with cytokinins (a) and pruning without biostimulants (b).

and if pruning plus urea is applied, the yield increases to 23.3 t ha^{-1}, equivalent to 38.7% more production. Banding branches is optional, since it is a potential pathogen entry point and can significantly affect the plants, for instance, gummosis and death caused by the *Lasiodiplodia theobramae* (Pat.) fungus (Valle *et al.*, 2019).

Regarding fruit quality, all the evaluated treatments were within the MX-FF-077-1996 quality norm parameters, such as equatorial diameters between 59.73 and 62.10 mm, juice percentages between 44 and 48% and total soluble solids between 9.0 and 9.6 °Brix. Statistical analyses of the fruit quality showed no significant differences between treatments, so biostimulants and practices applied to produce winter Persian lime had no effects on fruit quality (Table 1, 2 and 3); only significant differences between treatments were found for the polar diameter variable in the September experiment.

Biofol® is not a good option, as it increases production costs for flower induction, as well as calcium prohexadione application due to its high cost which does not represent a viable option; instead, the cytokinin-based product Citomax® showed the highest yield (30.3 t ha^{-1}) in Persian lime winter production, as reflected in the benefit-cost ratio economic analysis (Table 4).

CONCLUSIONS

Using biostimulants and agronomic practices to induce flowering and yield during winter in Persian limes at the state of Morelos, Mexico, were effective during July and September. Pruning and foliar urea application (6.0 kg/ha) had the best effects on winter Persian lime yield and their cost-benefit ratio. Cytokinin application (Citomax®) is a favorable option to achieve the best yields of Persian lime during winter. Fruit quality is not affected by the application of biostimulants and agronomic practices in the production of Persian lime during winter.

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Table 4. Economic analysis of experiment treatments using biostimulant and agronomic practices for floral induction and production of Persian lemon during the winter at the state of Morelos, Mexico, 2018/2019 (Taking a $10.00 /kg average rural price of Persian limes).

Treatment	CC	Yield	IB	IN	B/C	Rc
Pruning in july	85,000	15.6	156,000	71,000	1.8	0.8
Pruning in august	85,000	17.5	175,000	90,000	2	1
Pruning in september	85,000	17.5	175,000	90,000	2	1
Average pruning	85,000	16.8	168,000	83,000	1.9	0.9
Pruning + urea in july	95,000	21.7	217,000	122,000	2.3	1.3
Pruning + urea in august	95,000	25.8	258,000	163,000	2.7	1.7
Pruning + urea in september	95,000	22.6	226,000	131,000	2.4	1.4
Average pruning + urea	95,000	23.3	233,000	138,000	2.5	1.5
Pruning + urea + ringed in july	100,000	20.3	203,000	103,000	2	1
Pruning + urea + ringed in august	100,000	30.1	301,000	201,000	3	2
Pruning + urea + ringed in september	100,000	18.5	185,000	85,000	1.8	0.8
Average pruning + urea + ringed	100,000	22.9	229,000	129,000	2.3	1.3
Pruning + biofol® + ringed in july	97,000	12.4	124,000	27,000	1.3	0.3
Pruning + biofol® + ringed in august	97,000	19.9	199,000	102,000	2	1
Pruning + biofol® + ringed in september	97,000	15	150,000	53,000	1.5	0.5
Average pruning + biofol® + ringed	97,000	15.7	157,000	60,000	1.6	0.6
Pruning + Prohexadione calcium in september	167,000	17.6	176,000	9,000	1	0
Pruning + Citomax® in september	97,000	30.3	303,000	206,000	3.1	2.1

CC=Crop Costs, Yield, IB=Gross Index, IN=Net Index, B/C=Benefit (Cost) Ratio, Rc=Return on Capital.

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Colegio de Postgraduados

Alkaloids from the seed, calyx, and corolla of *Erythrina americana* Miller and *Erythrina coralloides* A.DC.

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ABSTRACT

Objective: To identify the main alkaloids present in the seeds, calyx, and corolla of *E. americana* and *E. coralloides* (Fabaceae) using HPLC-MS.

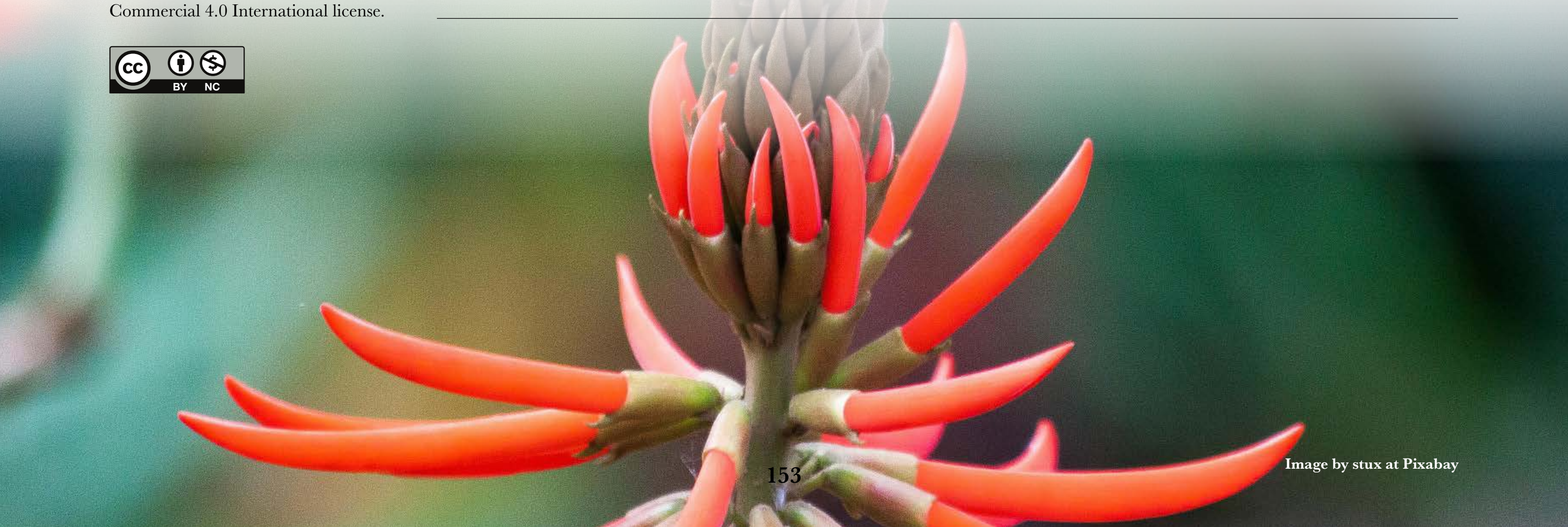
Design/methodology/approach: The seeds, calyx, and corolla of both species were separated; the crude alkaloid extracts were prepared according to the method described by Games *et al.* (1974). The crude alkaloid extracts were analyzed using a high-performance liquid chromatograph interfaced to a quadrupole ion trap mass spectrometer.

Results: The lactonic alkaloids -α-erythroidine and β-erythroidine were the main alkaloids found in *E. americana*, whereas the presence of erythrinine and 8-oxo erythraline is typical of *E. coralloides*. These compounds can be used to distinguish both species.

Limitations on study/implications: The use of the HPLC-MS technique allowed the detection of a large numbers of alkaloidal structures in different parts of the plants; however, this technique is not available in any laboratory.

Findings/conclusions: A large number of erythrinane-like alkaloids were found in seed, calyx, and corolla. The use of seeds and flowers in the preparation of diverse traditional dishes can cause soothing and sedating effects in consumers, as a result of the presence of lactonic alkaloids. The HPLC-MS method allowed the detection of alkaloidal structures in flowers and seeds that had not been identified in previous studies about this species.

Keywords: Alkaloids; chemotaxonomy; liquid chromatography-mass spectrometry.



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INTRODUCTION

The search for new active ingredients of natural origin based on the traditional use of certain plants is a well-documented fact. The genus *Erythrina* (Fabaceae) is one of the many Mexican plants used in traditional medicine. Several species of this genus are distributed throughout the world and a large number of these have been found in Mexico. In recent years the genus *Erythrina* was the subject of various researches throughout the world. Different aspects of the presence of flavonoids (Kumar *et al.*, 2013; Tanaka *et al.*, 2002; Chukwujekwu *et al.*, 2011), lectins (Turton *et al.*, 2004), or alkaloids (Garín-Aguilar *et al.*, 2005) have been studied. In particular, the following aspects of alkaloids have been the subject of study: structural analysis, biosynthesis (Maier *et al.*, 1999), pharmacology (Dantas *et al.*, 2004, Setti-Perdigão *et al.*, 2013, Saidu *et al.*, 2000), or chemotaxonomy (Yoshida *et al.*, 2009).

Previous studies have reported the presence of various alkaloids, but especially those that have an effect on the peripheral nervous system. These alkaloids include erysodine, an alkaloid isolated from the seeds of the genus *Erythrina*, which is an antagonist of neuronal nicotinic receptors $\alpha4\beta2$. This alkaloid was used to determine the role that these receptors play in memory consolidation (Garín-Aguilar *et al.*, 2009).

Two species of *Erythrin*, *E. americana* and *E. coralloides*, were studied to determine the alkaloid profile of their respective seeds and flowers (calyx and corolla), and therefore to compare their chemotaxonomic identity (Krukoff and Barneby, 1973).

MATERIALS AND METHODS

Seeds and flowers of *E. coralloides* and *E. americana* were collected in Texcoco and in Colorines, respectively; both towns are located in the State of Mexico. Specimens of each of the species were deposited in the CHAPA herbarium (Postgrado en Botánica, Colegio de Posgraduados). The authenticity of the specimens was certified by Mr. Joaquin Becerra Zabala from the CHAPA herbarium.

The flowers of both species were separated into calyx and corolla, and dehydrated in a Felisa FE-291 drying oven.

Crude extracts were prepared according to the method described by Games *et al.* (1974) for the extraction of alkaloids. Each plant material was extracted with hexane in a Soxhlet equipment for 48 hours. Vacuum evaporation was applied to the solvent and the residue was washed with 2% H_2SO_4; afterwards, the pH of the acidic phase was adjusted to 8 using $NaHCO_3$. Finally, extractions were carried out with CH_2Cl_2 (3x100), obtaining the hexane fraction of "free" alkaloids. The plant material was once more extracted with CH_3OH in the Soxhlet, the solvent was vacuum evaporated, and the residue was washed with 2% H_2SO_4. The acidic solution was extracted with CH_2Cl_2 (3x100) to remove traces of fat, and later adjusted to a pH of 8 with $NaHCO_3$, obtaining the methanolic fraction of "free" alkaloids.

The aqueous phase was acidified to a pH of 2 with HCl and refluxed at 60 °C for 3 hours to hydrolyze the esterified alkaloids. Then, the solution was adjusted to a pH of 8 and extracted with CH_2Cl_2 (3x100) to obtain the fraction of "released" alkaloids.

The alkaloid extracts were analyzed using the liquid chromatography-mass spectrometry (HPLC-MS) technique. For this purpose, the crude extract was analyzed using a Waters 600 high-performance liquid chromatograph, connected with a Finnigan LCQ mass spectrometer, using an atmospheric pressure chemical ionization (APCI) source. The alkaloids were separated by means of a 250 mm × 4.6 mm Superco Discovery C-18 (5um) column, at a 1 ml/min speed, using a linear mobile-phase with a programmed gradient of three solvents: A (0.1% ammonium acetate), B (Methanol), and C (acetonitrile) as follows: t=0 min, 75% A, 20% B, 5% C; t=10 min, 50% A, 45% B, 5% C; t=15 min, 50% A, 45% B, 5% C. The APCI source was vaporized at 450 °C; the nitrogen gas pressure was 80 and 20 psi respectively; and the capillary temperature was 150 °C. The alkaloids were confirmed and identified by means of mass spectrometry (MS), comparing the spectra with the authentic samples (standards) and with spectral libraries.

RESULTS AND DISCUSSION

Table 1 shows the crude extract amounts of *E. americana* and *E. coralloides*, expressed as mg of crude alkaloids/100 g of dry weight of each plant material. In the case of *E. americana*, the highest amount of crude extract was found in the seeds of the fraction of free alkaloids in methanol, while for *E. coralloides*, the highest fraction was found in the calyx of the fraction of free alkaloids in methanol.

The tissue analysis shows a high concentration of free alkaloids in CH_3OH and released alkaloids for both species; the concentration was lower for the fraction of free alkaloids in hexane. The hexane fraction was included because other studies (Hargreaves *et al.*, 1974; Sotelo *et al.*, 1993) have shown that it contains significant amounts of alkaloids. Some authors report that alkaloids represent 0.05 to 0.1% of the total plant of some species and that these substances were located in seeds, roots, bark, leaves, and flowers (Sotelo *et al.*, 1993; Dyke and Quessy, 1981); however, the concentration and type of alkaloids present in calyx and corolla have not been mentioned in any reports.

Table 2 shows that the erysodine (1), erythrinine (5), α-erythroidine (8), and β-erythroidine (9) alkaloids are distributed in both species; such alkaloids are characteristic of the species of the genus *Erythrina* found in the American continent. The erysovine (2) and erysopine (3) alkaloids were found only in the seeds of *E. coralloides*.

The erythraline (4), erythrinine (5), crystamidine (6), α-erythroidine (8), β-erythroidine (9), and 8-oxo-α-erythroidine (10) alkaloids were found in the *E. americana* calyx. The erythrinine (5), α-erythroidine (8), β-erythroidine (9), and 8-oxo-α-erythroidine (10) alkaloids were found in the corolla.

Table 1. *E. americana* and *E. coralloides* crude alkaloid fractions (mg/100 g dry tissue).

Specie	***E. americana* (mg)**			***E. coralloides* (mg)**		
Fraction	Calyx	Corolla	Seeds	Calyx	Corolla	Seeds
Free alkaloids in hexane	26	25	28	45	3.2	24.6
Free alkaloids in methanol	167.9	88	662	520	201	489
Liberatedalkaloid fraction	388	73	95	324	103.4	74

Table 2. Content and distribution of total alkaloids in *E. americana* and *E. coralloides*.

Specie	***E. americana***			***E. coralloides***		
Alkaloids	Calyx	Corolla	Seeds	Calyx	Corolla	Seeds
1.- Erysodine		*	*	*	*	*
2.- Erysovine						*
3.- Erysopine						*
4.- Erytraline	*		*	*	*	
5.- Erytrinine	*	*	*	*	*	*
6.- Cristamidine	*		*	*	*	*
7.- 8-oxo-erytraline			*	*	*	*
8.- α-erythroidine	*	*	*	*	*	
9.- β-erythroidine	*	*	*	*	*	
10.- 8-oxo-α-erythroidine	*	*	*	*		
11.- MW 289			*			
12.- MW 465					*	

The same alkaloids were found in *E. coralloides* calyx and corolla, with the exception of 8-oxo-α-erythroidine (10). The 8-oxo-erythraline (7) alkaloid was detected in the seeds of *E. americana* and in all the tissues of *E. coralloides*.

The structures found by means of this technique were diene (1-7) and lactonic (8-10) alkaloids. According to the spectra produced by HPLC-MS, an alkaloid with a PM of 289 (11) was observed in the seeds of *E. americana*, while an alkaloid with a PM of 465 (12) was found in the corolla of *E. coralloides*; these alkaloids had not been previously reported in these species. The HPLC-MS combination has the advantage of quickly and accurately determining the presence of alkaloids in a sample from a few milligrams. Likewise, the use of HPLC-MS technique allows the detection of a large number of alkaloidal structures in the calyx and corolla of the genus *Erythrina*. The use of their flowers in the preparation of traditional dishes benefits from this fact.

CONCLUSIONS

A large number of erythrinane alkaloids were found in seeds, calyx, and corolla. The use of seeds and flowers in the preparation of diverse traditional dishes can cause soothing and sedating effects in consumers, as a result of the presence of lactonic alkaloids. The HPLC-MS technique allowed the detection of alkaloids in the flowers and seeds of these species that had not been previously identified in other studies.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Mini grafts: alternative to obtain complete plants of *Pinus patula* propagated *in vitro*

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ABSTRACT

Micro-grafting and mini-grafting trials were carried out in order to obtain complete *Pinus patula* plants generated *in vitro*, derived from the fact that the formation of roots in micro-propagated shoots is very low or null. On the other hand, there is the possibility of generating clones with outstanding genetic characteristics. *P. patula* seeds were established in aseptic conditions and geminated *in vitro* in DCR medium; the aerial part was dissected from the base and roots were kept in DCR medium added with 0.5 mg l^{-1} of NAA to maintain them and served as mini rootstock, the shoots were transferred to DCR medium added with 2.0 mg l^{-1} of BA to form seedlings with an average of 3.9 shoots per explant at 30 days. On the other hand, 2-month-old nursery seedlings were used as rootstock for the mini-grafts. For the micro-graft tests, the shoots obtained were taken and the apical meristems with approximately 5 mm were isolated to conduct micro-grafting at the base with roots, and in the case of mini-grafts the shoots generated were allowed to grow to a length of 3 cm on average and grafted onto nursery seedlings. In both cases, the achievement of the grafted materials and the length were evaluated to measure the growth of the materials that had positive success. The response of micro-grafts was very low, 10% success, in addition to the manipulation of the meristems being very complex, which generated oxidation in the tissues. On the contrary, the mini-grafts showed 93.3 of grafting success and average growth of 26.05 cm, two months after the grafting process.

Key words: mini-graft, *in vitro* culture, micro-graft.

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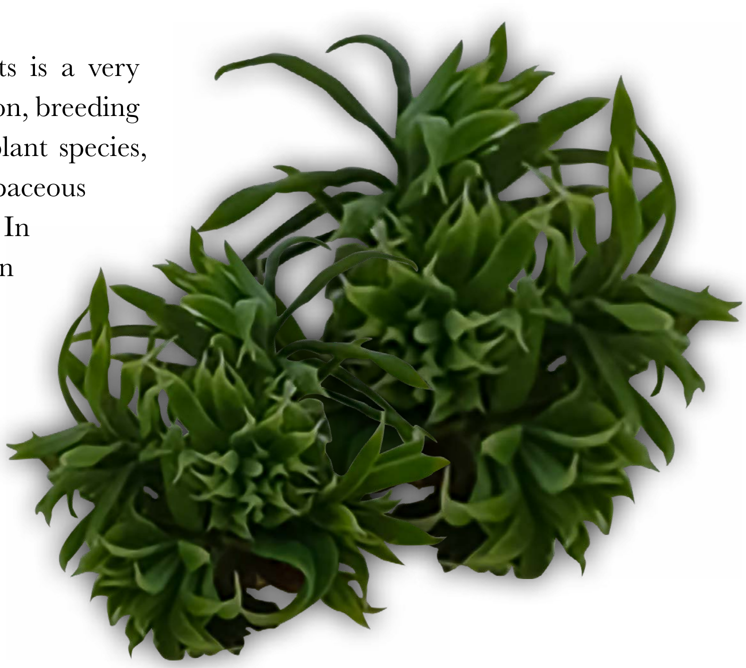
INTRODUCTION

In vitro propagation of plants is a very useful tool for massive propagation, breeding and conservation processes of plant species, used successfully in many herbaceous and some woody species. In conifers, responses have been reported in both organogenesis paths and in embryogenesis that have been very successful, since they guarantee generating complete plants (Atreea and Fowke, 1991; Grossnickle *et al.*, 1996).



There are very few species that present favorable responses in both organogenesis paths (organogenesis and embryogenesis) as *Pinus radiata* (Montalbán *et al.*, 2011; Prehn *et al.*, 2003). On the other hand, organogenesis in conifers has generated complete plants in few species, and the low or null formation of roots has been discouraging (Thorpe 2004); only few species have shown positive results, as is the case of *P. radiata* which has been able to be multiplied in vitro from young shoots (Prehn *et al.*, 2003). Propagating conifers *in vitro* opens the possibility of generating clones from selected trees and if the initial material is somatic tissue this is possible; for this reason, techniques such as micro-grafts have been explored, and successful protocols have been generated for *Pinus pinea* starting from mature shoots multiplied *in vitro* to be micro-grafted (Cortizo *et al.*, 2005). Another species with which studies have been performed in this regard is *Pinus radiata*; Fraga *et al.* (2002) found that in order to be successful the manipulation and quality of the micro-graft are very important. On the other hand, Oviedo *et al.* (2002) found that the time of the year is a defining factor that affects the success of micro-grafts. In the case of mini-grafts, this technique has been studied more in agricultural tree species such as citruses. An example of this is the research by Álvarez (2020) who evaluated terminal and inverted-T mini-grafts in *Citrus sinensis*; however, in forest species such as *Prosopis alba* the potential of this technique to produce this species in a large scale has been reported (Ewens and Felker, 2003). In this context, the aim of this study is to establish *Pinus patula* material in *in vitro* conditions, and to evaluate the grafting response both for micro- and mini-grafts.

MATERIALS AND METHODS

Biological material. The biological material used were *P. patula* seeds and nursery seedlings, in both cases from material collected in the state of Oaxaca. The seeds were germinated *in vitro* and the seedlings were kept in a greenhouse to be used as rootstock or understock. All the trials were carried out in the Forest Biotechnology laboratory of the National Center for Disciplinary Research in Conservation and Forest Ecosystem Improvement, located in Mexico City, Mexico. The *in vitro* management included four stages: Establishment in aseptic conditions and germination, Maintenance and multiplication, Rooting test, and Micro-graft tests. The mini-grafts tests are in fact, considered as *ex vitro* management.

Establishment in aseptic conditions and *in vitro* germination. The seeds were placed in a lidded tube or container with hydrogen peroxide at 3% v/v, during 24 h, previous to the *in vitro* germination. Then, the seeds were rinsed three times with sterile water to eliminate the peroxide. Following steps were carried out in a laminar flow hood, adding again hydrogen peroxide at 3% for 15 min, which it was then eliminated. Chlorine at 20% v/v was added for 12 minutes, and then eliminated through 5 times-rinses with distilled sterile water. At that point, the decontamination process of the seeds ends and they were placed in the DCR medium for germination; a total of 100 seeds were used in this process.

Maintenance and multiplication of *vitro* plants. The culture medium proposed by Gupta and Durzan (1985) known as DCR was used, supplemented with 30 g l^{-1} of sucrose, 1 g l^{-1} of activated carbon, and 9.0 g l^{-1} of agar. The pH was adjusted to 5.7 with a potentiometer (OAHUS), and the medium was sterilized in a semi-automatic vertical

Felisa autoclave for 15 minutes at 121 °C. For the multiplication of shoots, the medium was added with 2.0 mg l^{-1} of BA and, for the maintenance of the basal part with roots, with 0.5 mg l^{-1} of AIA. The incubation temperature was 26 °C, with a photoperiod of 16 h light and 8 h darkness, with luminous intensity of 68 μmol m^{-2} s^{-1} using white light LED lamps. They were allowed to grow for 30 days.

Rooting tests in the *in vitro* culture. As the main objective of the study was to generate complete plants, a rooting test was conducted under *in vitro* conditions. 30 shoots were used to test three doses 0.5, 1.0 and 2.0 mg l^{-1} of ANA, 2% of activated carbon (Sigma®), added to the DCR base medium. We evaluated root formation at day 30th. The results of this test were taken as negative control for the evaluation of micro- and mini-grafts.

Micro- and mini- grafts trials. For the micro-graft trials, the shoots obtained from *in vitro* multiplication were taken and the apical meristems with approximately 5 mm were isolated. With the help of a stereoscopic microscope, micro-grafting was done on the base of roots, the entire process was carried out in a laminar flow hood (*in vitro* manipulation). For the mini-grafts, the shoots generated were allowed to grow to an average length of 3 cm and were grafted in two-month-old nursery seedlings with an average length of 26.7 cm (terminal graft was performed). This process is considered *ex vitro* management since it is not done in the laminar flow hood and there is no strict control of asepsis.

Statistical analysis. All trials were performed under a completely random experimental design with 30 repetitions per treatment. Recorded variables were graft success for both micro and mini, explant length, number of internodes, number of shoots and roots. Two evaluations were done, at 30 and 60 days. As control for complete plants (positive control), data from germinated seeds were taken and 30 shoots were used as negative control in medium supplemented with 0.5, 1.0 and 2.0 mg l^{-1} of AIA. Analysis of variance was performed with the data obtained, and means comparison was done with Tukey's test ($P \leq 0.05$). The SAS v9 software was used for data analysis (SAS Institute, 2002).

RESULTS AND DISCUSSION

The *in vitro* establishment of *Pinus patula* was achieved successfully from seeds, taking as a reference the results reported by Castillo *et al.* (2018) in *Pesudotsuga menziesii*. A germination of 96% was obtained in aseptic conditions and only 2.8% of contamination, so it is considered that the decontamination train was efficient.

The separation of the hypocotyl to be used as initial explant for the multiplication, in DCR medium added with BA, had de result of the formation of de *novo* shoots with an average of 3.9 shoots per explant, as reported by Arnold and Eriksson (1981) in *Pinus contorta* or what was observed in *Pesudotsuga menziesii* by Castillo *et al.* (2018). Although it is not a high multiplication rage, let us remember that it is exponential to the extent that sub-cultures are carried out.

Concerning the isolated epicotyls to maintain the base and the roots, it was achieved for these to be maintained for a period of 30 days, conserving their vigor in DCR medium with 0.5 mg l^{-1} of AIA; these served as micro-rootstock or micro-understock, for the micro-grafting process.

The formation of roots in shoots generated *in vitro* has been reported in other species such as *Pinus massoniana*, through the addition of ANA to the culture medium (Wang and Yao, 2019), although a good part of species of the genus *Pinus* generate a low or null number of roots (Thorpe, 2004). The tests performed with three concentrations of ANA added to the DCR medium showed this response with *Pinus patula* (Table 1), and therefore in the micro- and mini- grafting tests the seedlings that formed a low number of roots were considered as negative control, and on the other hand, the data of seedlings derived from the *in vitro* germination as positive control.

Regarding the micro-grafts trials, contrary to what was reported in other species —such as *Pinus pinea*, which reported 43% of success, and *Pinus radiata* (Fraga *et al.*, 2002 and Oviedo *et al.*, 2008) which reported successful grafting, growth and development of micro-grafted plants— and to what was expected, it was observed that even with experienced staff every micro-graft took on average 36 minutes, due to the complexity of the process itself and therefore the excessive manipulation time of meristems dissected to be micro-grafted. At the end of the evaluation, low grafting success and oxidation of tissues took place (Table 2).

In the case of mini-grafts (*in vitro* shoots generated, used as spikes and seedlings produced from seed as rootstock or understock), according to those reported in other species such as *Prosopis alba*, which through mini-grafts achieved a maximum of 70% of grasping indicate that the technique for massive production and at the scale of plants can be used (Ewens and Felker, 2001). In this sense, grafting success with high percentage and terminal graft technique was achieved, done in a time of 4 minutes on average, including the cutting, generation of the graft's draw cleft, and protection with union cite. Contamination was not observed and 93.3% of success was achieved (Table 2 and Figure 1).

Table 1. Effect of three ANA concentrations on the formation of *in vitro* roots in *Pinus patula*.

Trial ANA	Root Length (mm)	Number of Roots
Testigo	12.60 a	7.03 a
0.5	1.56 c	1.28 b
1.0	2.36 b	0.94 bc
2.0	1.95 bc	0.63 c

Means with the same letter in columns are statistically equal (Tukey, 0.05). The control was germinated *in vitro*.

Table 2. Grafting response of micro- and mini-grafts in *Pinus patula* at 30 days.

Trial	Growth Length mm	Performance	Whole Plant	Number of roots
Control +	33.60 c	N/A	Si	21.03 a
Control −	6.56	N/A	No	1.28 c
Micro graft	2.36 b	3.0	Si	1.94 b
Mini graft	26.05 b	28.0	Si	18.63 ab

Means with the same letter in columns are statistically equal (Tukey, 0.05), the Control + correspond to nursery seedlings, the control − corresponds to shoots obtained *in vitro* in rooting medium.

Figure 1. a) *In vitro* development of *Pinus patula* shoots at 30 days in DCR medium added with BA, b) Grafting success and development of mini-graft at 30 days, c) Union of rootstock and graft generated *in vitro* of *Pinus patula*.

CONCLUSIONS

The establishment of *Pinus patula* was achieved in *in vitro* conditions. Germination was obtained from seeds in DCR medium, and a multiplication of shoots in this medium added with 2.0 mg l^{-1} of BA.

The management of micro-grafts for *Pinus patula* showed a high degree of complexity in the isolation and management of meristems and therefore, in the process of micro-grafting, where there were oxidation problems and low success.

The technique of mini-grafts is a viable option for *Pinus patula*, of simple management with an acceptable grafting success, growth and development of mini-grafts.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effect of pruning on *Jatropha curcas* L. seedlings during the nursery stage

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ABSTRACT

Objective To evaluate the effect of pruning on seedlings of *Jatropha curcas* L. during the nursery stage.

Design/Methodology/Approach. To determine the effect of pruning on *Jatropha curcas* L. seedlings during the nursery stage, a study was carried out at the nursery of the CPA "Abel Santamaría" of the Martí municipality, Cuba. A complete randomized design was used. Three treatments (T) were tested: 1) control (without pruning), 2) pruning 30 cm above the soil and seedling defoliation, 3) pruning 30 cm above the soil and without seedling defoliation. A descriptive analysis was carried out to determine the survival, regrowth, and number of plants with primary branches. For the number of leaves and branches, height, and diameter, a simple analysis of variance was used, with partitions for each observation.

Results. No treatment influenced the survival and regrowth of the plant: up to 2 branches of T2 and T3 were obtained per plant. A greater height was recorded with T1 (38.63 cm) and this result is different from T2 and T3. The highest number of branches that formed primary branches was obtained by pruning and without defoliation (28 *vs.* 22 and 0, for T2 and T1, respectively).

Study Limitations/Implications. Pruning is an option that can increase *Jatropha's* agronomic yield, but it has not been studied under nursery conditions.

Findings/Conclusions. The pruning of *J. curcas* seedlings at 30 cm above the ground, under nursery conditions, with or without defoliation, does not affect the survival or regrowth of the crops and the development of the primary branches. However, more plants with primary branches and a wider stem diameter can be obtained by pruning without defoliation.

Keywords: defoliation, survival, regrowth, biofuels.

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INTRODUCTION

Jatropha curcas (L.) is a shrub of the Euphorbiaceae family, native to Central America. Its seeds can contain up to 35% oil, which can be processed to produce high-quality raw material for biofuels and specialized products (García *et al.*, 2017).

This monoecious plant has male and female flowers in the same inflorescence and sporadically develops hermaphrodite flowers. Generally, the inflorescence is a terminal panicle: the female flowers are located in the center of the inflorescence and the male flowers, in the periphery (Dasumiati *et al.*, 2017). In addition, the panicles are only found at the terminal ends of the plant. Therefore, the yield of the harvest will depend on the number of terminals per plant. Pruning is very important and plays a fundamental role in the production of flowers and the subsequent fruit yield (Senger, 2018).

Pruning in *Jatropha* allows plants to have a greater number of branches and a proportionally higher production, allows a good penetration of the solar radiation, facilitates the passage of the wind, strengthens the productive branches, regulates the size of the plants, facilitates the manual collection of fruits, and eliminates damaged or unproductive branches (Muñoz *et al*., 2016; Lamonarca, 2017).

The studies about the subject fundamentally refer to the effect of pruning during the establishment and production phase of the plant (Díaz, 2015). Consequently, determining the morphological response during the nursery phase should be one of the first research areas; this is very important for the subsequent management of productive plantations.

Therefore, the objective of this work was to evaluate the effect of different pruning practices on *Jatropha curcas* L. seedlings during the nursery stage.

MATERIALS AND METHODS

The study was carried out in the nursery of the Abel Santa María CPA, located in the municipality of Martí, Matanzas province, Cuba, from March to June 2014. During the study period, a 376.4-mm rainfall and 23.9 °C mean temperature were recorded.

We used 20 cm × 12 cm perforated black polyethylene bags, which were filled with a mixture of soil (70%) and organic matter (30%).

Design and treatments: A complete randomized design was used. Thirty repetitions were evaluated per treatment. Three treatments were studied: 1) control (without pruning), 2) pruning at 30 cm above the soil and seedling defoliation, 3) pruning at 30 cm above the soil without seedling defoliation.

Experimental procedure: Two seeds were sown per bag, using the "Cabo Verde" provenance harvested at the "Indio Hatuey" Estación Experimental de Pastos y Forrajes. All the material planted in the nursery received a daily morning irrigation (4 L/minute/m^2) to guarantee the emergence and development of the seedlings. When they reached an average height of 7 cm, thinning was carried out to select the most vigorous seedling per bag.

When the seedlings reached a height of 60-70 cm, 60 days after sowing, they were pruned according to the indicated treatments.

Registered variables: To evaluate survival, regrowth, and the number of new leaves, weekly samplings were carried out seven days after the pruning. One-hundred twenty days after sowing optimal seedlings were transplanted to the field. Overall, eight measurements were made during the period. The number of plants that formed primary branches and the number of branches were evaluated, based on visual counting, from the second observation.

A measuring tape was used to determine the height: *i.e*., the distance from the base of the stem (ground) to the end of the highest branch. A vernier caliper was used to measure the thickness of the seedlings in the main stem at a height of 20 cm (Campuzano, 2009). Both measurements were made from the third week after pruning.

Statistical Analysis: A descriptive analysis was carried out for the survival, regrowth of plants, and number of plants that formed primary branches indicators; meanwhile, the number of leaves, new branches, height, and thickness were subject to a simple analysis of variance with partitions for each observation. The compliance with the homogeneity of variances and normal distribution assumptions were first verified. The Infostat statistical package, version 1.1, was used. The means were compared using Duncan's test (1955) with a $p \leq 0.05$ significance level.

RESULTS AND DISCUSSION

Figure 1 shows the survival of the seedlings by pruning effect. No treatment was determined to influence this variable; one hundred percent of the study plants survived with all treatments in each observation. This result indicates that the response was independent of the evaluated management.

It has been determined that pruning has had a variable effect on the survival of other trees. For example, in a study carried out on *Nothofagus nervosa* by Donoso *et al.* (2009), 82% survival was obtained in the first year when apical cuts were applied. However, Guacín (2014) demonstrated that, when this technique was applied to *Prosopis* sp., there was no mortality among the trees.

In the case of *J. curcas*, no studies related to pruning during the nursery phase were found. However, the objective of this practice is to guarantee the development of more branches for fruit production and to shape the structure of the plant from an early age. Therefore, establishing that this agricultural management did not affect survival was of great importance.

Figure 2 shows the effect of pruning on the regrowth of seedlings. For this variable, no significant differences were found between treatments. The applied technique did not have a determinant effect on the regrowth of any of the plants.

This result was different from the findings of Bacab *et al.* (2012) for other trees such as *Leucaena leucocephala*, in which pruning had a significant effect on regrowth.

South (2016) recognized that pruning young plants leaves them more vulnerable to stress, as a consequence of the removal of biomass. In addition, South argues that, if seedlings survive, they will require large amounts of resources to recover (regrowth). When this criterion is compared with the results obtained in this study, only contradictory speculations can be made. The specific response of this species shows no evidence of handling-induced stress; the various pruning techniques did not affect the behavior of the

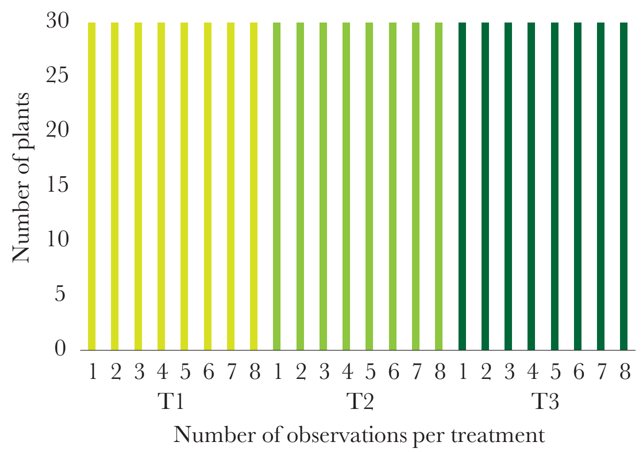

Figure 1. Effect of pruning on seedlings survival.

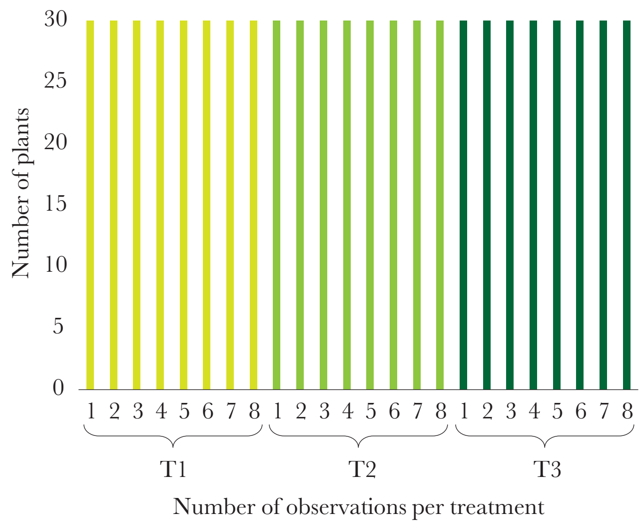

Figure 2. Effect of pruning on the regrowth of seedlings.

plants. However, studies about the subject are deficient or null; therefore, providing a crop-based physiological explanation is difficult.

During the whole study, the plants did not stop growing new leaves (Table 1). Significant differences were found at each moment between the evaluated treatments ($p<0.05$). However, the response was variable in each observation. From the first to the third week, treatment 3 grew the highest number of leaves; in week 4, it did not statistically differ from treatment 2; afterwards, the latter recorded the greater number of leaves during

Table 1. Behavior of some morphological variables after pruning.

Variables	**Treatments**	**Sampling**							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
leaves (number)	1 2 3	3^{c} 4^{b} 5^{a}	5^{c} 6^{b} 7^{a}	6^{c} 7^{b} 8^{a}	7^{b} 8^{a} 8^{a}	7^{c} 9^{a} 8^{b}	7^{c} 9^{a} 8^{b}	7^{b} 9^{a} 9^{a}	7^{b} 9^{a} 9^{a}
Standard error (+)		0.33*	0.50*	0.50*	0.53*	0.50*	0.44*	0.44*	0.40*
branches (number)	1 2 3	- - -	0^{c} 2^{a} 1^{b}	0^{c} 2^{a} 1^{b}	0^{c} 2^{a} 1^{b}	0^{c} 2^{a} 1^{b}	0^{b} 2^{a} 2^{a}	0^{b} 2^{a} 2^{a}	0^{b} 2^{a} 2^{a}
Standard error (+)		-	0.12*	0.07*	0.09*	0.08*	0.11*	0.06*	0.08*
Height (cm)	1 2 3	- - -	- - -	28.40^{a} 16.47^{c} 21.71^{b}	31.30^{a} 18.17^{c} 24.70^{b}	32.45^{a} 22.51^{c} 26.10^{b}	33.93^{a} 26.77^{c} 27.10^{b}	36.00^{a} 27.90^{c} 28.53^{b}	38.63^{a} 29.30^{b} 29.57^{b}
Standard error (+)		-	-	1.04*	1.16*	1.05*	1.14*	1.11*	1.05*
Stem thickness (cm)	1 2 3	- - -	- - -	0.80^{b} 0.83^{b} 0.94^{a}	0.80^{b} 0.83^{b} 0.94^{a}	0.85^{b} 0.88^{b} 0.97^{a}	0.86^{b} 0.88^{b} 0.97^{a}	0.86^{c} 0.90^{b} 0.99^{a}	0.90^{b} 0.94^{b} 0.99^{a}
Standard error (+)		-	-	0.03*	0.03*	0.05*	0.05*	0.03*	0.04*

Means with a common letter are not significantly different ($p\leq0.05$). ± standard error (E st).
Average with a common letter is not significantly different ($p\leq0.05$).

the observations corresponding to fifth and sixth weeks. Subsequently no differences with respect to treatment 3 in the penultimate and last weeks were recorded; therefore, both techniques have a positive influence in the development of new formed leaves, since unpruned plants were the ones in which this variable was least developed.

Walteros *et al.* (2013) found similar results by pruning young *Vitis vinifera* plants; they were able to stimulate the growth of biomass in the aerial part. Guacín (2014) also highlighted the positive effect of pruning on the formation of leaves and shoots of *Prosopis* sp., since the first weeks of the crop. According to Basave *et al.* (2014), not all species respond to pruning techniques, since growth and branching patterns vary from one plant family to another.

The highest number of branches formed was remarkable from the second week in the plants to which pruning and stripping (treatment 2) were applied and it differed significantly ($p<0.05$) from the rest of the treatments until the sixth week. Subsequently, for observations 7 and 8, no significant differences were found in the plants that were pruned, but not defoliated. For both treatments, up to 2 branches per plant were formed at the end of the nursery stage (week 8). Unpruned plants (treatment 1) did not grow branches during the evaluation time.

Meanwhile, these results corroborate the criteria of Riikonen and Luoranen (2018) by stating that the number of leaves in a tree are partially the result of the presence of primary branches —a characteristic that was observed in this study. The treatments with the highest number of primary branches were also those with the highest number of leaves.

The lack of pruning (treatment 1) resulted in the highest plants (Table 1) in each observation and the height differed significantly from the rest of the treatments.

At the end of the evaluation period, the unpruned plants reached an average height of 38.63 cm, taller than the pruned plants, which reached 29.30 and 29.57 cm, for treatments 2 and 3, respectively.

Umeki *et al.* (2018) consider that this is a normal physiological behavior, because —when the apical shoots of the plants are cut— the “apical dominance” stops and a stimulation of cell division and a higher concentration of nutrients and enzymes in the lateral branches take place.

In each of the observations, significant differences in stem diameter (Table 1) were also found between treatments. It was notable that treatment 3 —in which pruning was applied, but no defoliation took place— always obtained the thickest stems.

These results do not match those obtained by Hoyos and Hurtado (2017) for the cultivation of the papaya (*Carica papaya*), which show that unpruned plants had a wider stem diameter. The difference in the response could have various causes: different plant families, the effect of the genotype-environment interaction, the handling or the applied techniques, among others. Nevertheless, the positive response to this variable is known to be of great importance for all species, since it translates into greater nutrient reserves that guarantee the development and growth of the crop (Basave *et al.*, 2021)

Determining which of the applied techniques provided the highest number of plants that formed primary branches (Table 2) was an important measure. Establishing the proper handling that gradually shapes the productive structure of *Jatropha curcas* in the nursery stage will guarantee that other pruning can be carried out during the establishment and

Table 2. Number of plants that formed primary branches according to the effect of pruning.

Treatment	Number of plants that formed primary branches
1	0
2	22
3	28

development phase of the crop. Therefore, the number of branches can proportionally increase with regard to a greater production of fruits per plant.

Table 2 shows that, by pruning and not defoliating (treatment 3), more plants with primary branches (28 *vs.* 22 and 0) were formed. This is perhaps related to the presence of the leaves that carry out the photosynthesis process. This could have favored the elongation of the shoots that subsequently became branches (Chávez *et al.*, 2017).

In general terms, the present study verified the positive response of *Jatropha curcas* when it is pruned during the nursery stage. All the plants survived the handling; it was possible to start the conformation and development of primary branches from an early age, without affecting the biological cycle of the crop.

CONCLUSIONS

The results of this assessment showed that *Jatropha curcas* seedlings can be pruned during their nursery stage, 30 cm above the ground, with or without defoliation techniques; neither impacts the survival or regrowth of the crop and the development of primary branches. However, more plants with primary branches and a thicker stem were obtained, when they were pruned, but not defoliated.

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AP AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Pruning and fertilization in young *Pinus greggii* plantations established at Durango, Mexico

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ABSTRACT

Objective: To evaluate the effects of pruning and fertilization on the growth of *Pinus greggii* plantations established at Durango state, Mexico.

Design/methodology/approach: In a plantation (Durango), pruning was applied in three intensities (0, 50, and 75%) in a randomized complete block design. Four years later, the basal diameter (Db), normal diameter (ND), total plant height (PH), and stem height (SH) were evaluated. In another plantation (La Soledad), two pruning levels (0 and 50%) and foliar fertilization (with and without application) were applied in a completely randomized factorial design. Seven months later, Db, PH, and crown diameter (CD) were assessed. The analysis of their variance and means comparison test (Tukey, $\alpha=0.05$) were performed.

Results: In Durango, pruning diminished the Db and PH, but increased the SH ($p\leq0.01$). In La Soledad, pruning also affected growth. The Db, PH, and CD were lower ($p\leq0.01$) when trees were pruned. Fertilization affected the PH ($p\leq0.05$) and CD ($p\leq0.01$) as well. The PH and the CD for both pruned and unpruned trees increased with fertilization.

Limitations/implications: Pruning at 50 and 75% intensities are not suitable for *P. greggii* because it reduces their growth.

Findings/conclusions: Fertilization favors the growth of *P. greggii* planted on low fertility soils for both pruned and unpruned trees, but the pruning and fertilization interaction should be examined in detail for a longer period.

Keywords: biomass, industry, forest products, productivity, silviculture.

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INTRODUCTION

In Mexico, establishing commercial forest plantations has acquired great relevance during the last decade (Fierros González, 2012). This, as a strategy to increase the productivity of the forest sector and reduce the Mexican national deficit of forest products, which reaches 66% (Llano & Fernández, 2017). The state of Durango is part of this strategy and in 2017 it was proposed to establish 1,000 hectares of commercial forest plantations with various species of pine (National Forestry Commission [CONAFOR], 2017), one of them *Pinus greggii* Engelm. ex Parl. ("pino Prieto"), one of the main species used in this type of production system (Fierros, 2012). *Pinus greggii* is preferred due to its high growth rate

Image by Jeon Sang-O at Pixabay

coupled with its high potential to establish in limited humidity conditions, which allows it to thrive in marginal sites where other pine species are difficult to establish (Domínguez *et al*., 2001; Gómez-Romero *et al*., 2012).

Forest plantations require adequate silvicultural management that increases tree's growth rate because technical shifts are usually long (CONAFOR-Colegio de Postgraduados [CP], 2011). The long period for the plantations profitability onset delay obtaining economic perceptions, which discourages new plantations establishment and limits the continuity in the management of those already established. Therefore, it is a priority to accelerate the growth of commercial *P. greggii* plantations established in marginal agricultural lands, with viable and sustainable silvicultural alternatives, which in turn increase the biomass quantity and quality.

Pruning is a silvicultural practice used in forest plantations to stimulate and regulate the growth of trees, as well as to maintain the shape and density of their crown, ensuring a greater stem height, clean and free of knots (Braz *et al*., 2017; Ferrere *et al*., 2015; Ferraz *et al*., 2016). However, one of the main factors on which its effectiveness depends is the intensity with which it is carried out (Davel, 2013; Erkan *et al*., 2016). High pruning intensities in young *Pinus brutia* Ten. plantations (50 and 75%) decreased tree growth (Erkan *et al*., 2016). Also, in the plantations of *Tectona grandis* L. f., pruning of almost 50% improved growth and production of knot-free wood (Víquez and Pérez, 2005). The effects of pruning are related to its influence on the production/balance rates of endogenous growth regulators, mobilization and redistribution of carbohydrates and nutritional reserves; as well as, with photosynthetic efficiency (Stiles, 1984), all of the physiological processes that can be improved if this silvicultural managing is properly carried out.

Fertilization is another important silvicultural practice in forest plantations management (Smethurst, 2010). Constant availability of nutrients contributes to favorable growth, especially in marginal soils, such as those that predominate the areas where forest plantations of *P. greggii* are established in Durango. In an early fertilization trial, it was reported that fertilization favored *P. greggii* growth in low-productive sites (Vázquez-Cisneros *et al*., 2018). The benefits of fertilizing are also reported in a *Pinus cooperi* C. E. Blanco plantation, in which seedlings improved their growth with the phosphorus application, as this is the most deficient element (Hernández *et al*., 2018). The above suggests that fertilization can favor the growth of *P. greggii* plantations at Durango. The objective of this research was to evaluate the effect of pruning and foliar fertilization on *P. greggii* growth in plantations established at Durango.

MATERIALS AND METHODS

Plantation at Durango state

An experimental agroforestry plantation of *P. greggii* was evaluated in 2020 as a preliminary model of commercial plantations. This plantation was established in 2007 as a provenance test and subsequently treated as a Christmas pine plantation until its management was possible. The plantation had an initial density of 2,222 trees/ha, with 3 m spacings between rows and 1.5 m between trees. The experimental site was located at 23° 59' 27" N, 104° 37' 30" W, and an altitude of 1,881 m a.s.l. The predominant soil in the site is loamy (clay and sandy), with intermediate moisture retention capacity, medium depth, 0 to 2% slope, 7.9 pH, and low in organic matter content (<1.5%) and nutrients. The climate at the study site is temperate semi-arid [BS_1 kw (w) (e)], with a rainy season in summer and an annual mean temperature of 16.3 °C. The accumulated rainfall during the year reaches an average of 476 mm, with maximum precipitation between June and September (Medina *et al.*, 2005).

In September 2016, three pruning treatments were applied: 0 (no pruning), 50 and 75%, under a randomized complete block design with four repetitions and a useful plot of 40 trees (observations) per repetition. Pruning was carried out at the lower part of each tree's crown with appropriate tools (pruning shears and saw) to make clean cuts, close to the trunk and without leaving a stump. Immediately after pruning, the normal diameter (ND) and total plant height (PH) were evaluated, and later in March 2020, the basal diameter (Db), ND, PH, and stem or trunk height (SH) were assessed.

A caliper was used to measure Db 10 cm from the root crown and ND at 130 cm from uniform stems. The plant and stem height were evaluated with a telescopic metric ruler. The PH was measured from the soil surface to the main stem apex, the SH (which is the commercially important part of the stem) from the soil surface at the beginning of the crown. The data obtained were used for the analysis of variance and in the variables that reported significant differences between treatments, based on an $\alpha=0.05$ value, a means comparison test was carried out on them (Tukey; $p\leq0.05$).

Plantation at La Soledad, Canatlán, Durango.

A commercial plantation of *P. greggii* established in agricultural soil during 2015 was evaluated. It had a density of 1258 plants/ha (3.00 m between rows and 2.65 m between plants). In trees with similar height and diameter, two pruning treatments (0 and 50%) and foliar fertilization (without and with application) were applied in 2020. The pruning was carried out on February 23, 2020, just as it was pruned at the Durango, Durango plantation. At 12 and 19 days after pruning, fertilization treatments were applied by spraying foliar fertilizer (Bayfolan®, 1.1 L / ha), with a manual pump. The soil was a sandy-loam texture, low organic matter content (1.1%), 14.3% field capacity, 8.3 pH, medium to low levels of nitrogen (11.1 mg/kg), phosphorus (18.8 mg/kg) and potassium (582.7 mg/kg). A completely randomized experimental design was used in a factorial arrangement of treatments (2×2) and two repetitions with nine trees each.

The basal diameter (Db) of the stem, total plant height (PH), and crown diameter (CD) were evaluated immediately after pruning and for seven months. For this, nine uniform stems were used per repetition, in which the Db was determined with the caliper at 10 cm from the soil surface. The PH was determined with a telescopic metric ruler and CD with a tape measure. The PH was evaluated from the soil surface and up to the main stem apex. The CD was measured in two directions (east-west and north-south), to later obtain the mean value used for the analysis of variance. With the obtained data, a statistical analysis was carried out according to the used experimental design. In case of statistical differences (value of $\alpha=0.05$), for the main or interaction effects, a means comparison Tukey test ($p\leq0.05$) was done in the SAS software version 9.2® (SAS, 2009).

RESULTS AND DISCUSSION

At the Durango plantation, the trees did not statistically differ in their ND and PH values at the time of pruning ($p>0.05$). The initial ND and PH were 0% pruning = 6.8 cm and 4.0 m; 50% pruning = 6.7 cm and 3.8 m; 75% pruning = 7.1 cm and 4.0 m respectively. This reference served as the basis for evaluating the effect of pruning treatments on trees with uniform initial growth, and thus, avoiding bias when analyzing the effects in the subsequent evaluation. In 2020, pruning significantly affected ($p\leq0.01$) Db, PH, and SH. The highest Db was recorded in trees with no pruning (15.4 cm), this was 9% higher than the registered value (14.1 cm) in 50% of pruned trees (Table 1). Likewise, the PH was higher in non-pruned trees (6.8 m) and exceeded by 15% the 5.9 m value registered in the 75% pruning treatment (Table 1). Finally, the SH was 13 times higher in pruning at 75% (2.6 m) compared to the lowest SH (0.2 m) reported in non-pruned trees (Table 1).

La Soledad, Canatlán, Durango state

Pruning significantly affected the Db, PH, and CD ($p \leq 0.01$) variables. Pruning reduced the Db of the trees by 1.2 cm when comparing the mean 7.8 cm value in the treatment with no pruning to the 6.6 cm of the 50 % pruned trees (Table 2). The PH had a 25 %superiority in the treatment without pruning (231.6 cm) in relation to the pruned trees (185.9 cm) (Table 2). The CD was significantly higher in the treatment with no pruning (168.1 cm), more than double when compared to the pruned ones (88.0 cm) (Table 2). Likewise, fertilization statistically affected PH and CD ($p \leq 0.05$ and $p \leq 0.01$, respectively). Fertilization favored the height growth in both pruned and unpruned trees (Figure 1). The PH increased 4 % in pruned trees and 25 % in non-pruned trees (Table 2). With fertilization, CD increased 21 % in pruned trees and 13 % in non-pruned trees (Table 2).

Pruning in commercial forest plantations is considered a silvicultural practice that favors tree growth (Erkan *et al.*, 2016; Víquez & Pérez, 2005) but the results with *P. greggii* do not support this in any of the evaluation sites. None of the analyzed intensities favored growth in height or diameter. Pruning only increased the height of the stems (reduction of the living crown ratio), due to the removal of the basal branches. Similar results were reported in young *Pinus brutia* plantations, in which 50 and 75% pruning intensities decreased the growth in the diameter of the trees (Erkan *et al.*, 2016). In a plantation of

Table 1. Average values of the evaluated variables in a *Pinus greggii* plantation established at Durango, Durango state, Mexico. 2020.

Pruning treatment	Basal diameter (cm)	Normal diameter (cm)	Total height (m)	Trunk height (m)
0%	15.4^{a}	9.8	6.8^{a}	0.2^{c}
50%	14.1^{b}	9.3	6.1^{ab}	1.4^{b}
75%	15.1^{ab}	9.6	5.9^{b}	2.6^{a}
Average	14.8	9.5	6.3	1.2
[1]CV (%)	23.8	29.1	20.6	21.5

[1]CV=coefficient of variation. Letters in each column represent significant differences between pruning treatments (a-c) (Tukey $p\leq0.05$).

Table 2. Averages of the evaluated variables at a *Pinus greggii* plantation with two pruning and fertilization treatments. La Soledad, Durango, Mexico. 2020.

Treatment	Fertilization	Basal diameter (cm)	Total height (cm)	Crown diameter (cm)
With pruning	Without fertilization	6.7	182.4^{c}	79.8^{d}
	Foliar fertilization	6.5	189.3^{c}	96.2^{c}
	Average	6.6^{B}	185.9^{B}	88.0^{B}
Without pruning	Without fertilization	7.1	205.9^{b}	157.7^{b}
	Foliar fertilization	8.4	257.3^{a}	178.5^{a}
	Average	7.8^{A}	231.6^{A}	168.1^{A}
[1]CV (%)		23.3	22.5	22.9

[1]CV=coefficient of variation. Letters in each column represent significant differences between pruning (A-B) and fertilization treatments (a-d) according to Tukey's test (p≤0.05).

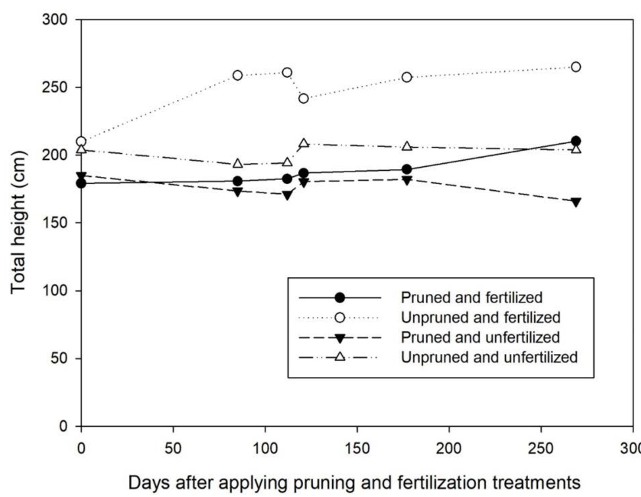

Figure 1. Responses of *Pinus greggii* trees to treatments of pruning and fertilization in a commercial forest plantation established in La Soledad, Dgo.

Eucalyptus grandis × *Eucalyptus urophylla* clones in Brazil, tree growth was also reduced with 40 and 60% pruning intensities (Ferraz *et al.*, 2016). Yet, in this same plantation, a 20% pruning intensity improved growth, so that it is deduced that each species has a level at which pruning has favorable and not detrimental effects if properly implemented (Braz *et al.*, 2017). So, the analyzed pruning intensities in *P. greggii* were probably not the most convenient. In *P. greggii* it is suggested to analyze intensities of less than 50% to define if there is an appropriate level.

In *P. greggii*, pruning is necessary to increase the main stem height, which is the commercial part of the tree, since the species tends to branch almost from the base of the stem, as observed in the unpruned trees of the Durango plantation. Also, pruning has economic implications (Huang & Kronrad, 2004), therefore, its implementation must be beneficial for tree growth.

In natural stands, natural pruning is a mechanism that favors tree growth by allowing more light to enter the different canopy strata (Musálem & Fierros, 1996). This happens when crown competition is high so that natural pruning can be promoted by maintaining high tree density (Musálem & Fierros, 1996). Although artificial pruning was used in the assessed plantations, there was no favorable response in the tree's growth due to the influence of the species and other factors such as humidity, nutrients, and planting density that could restrict growth. The density of the plantation was especially high in Durango, compared to the densities recommended for most forest plantations with conifers, close to 1100 trees/ha (CONAFOR-CP, 2011). The pruning effectiveness has been linked to thinning (Ferrere *et al.*, 2015), which is the main silvicultural operation with which the density of the trees is manipulated, both in commercial plantations and natural stands, with repercussions on the growth and wood quality (Ramos *et al.*, 2014; Vásquez-García *et al.*, 2016).

The pruning intensities evaluated in this study were high and ended up damaging the tree's growth rate. Musálem & Fierros (1996) suggest that the lower branches can be pruned because they contribute little to photosynthesis and tend to present a high expenditure of photosynthate for maintenance. However, it should be considered that in the 50 and 75% pruning, photosynthetically active tissue was eliminated, which limited photoassimilates production and, therefore, the number of resources for growth. In addition, there may be competition in photoassimilates usage to satisfy other demands, such as defense (Lambers *et al.*, 2008), to heal and cover the stumps left by the branches after pruning. In *Pseudotsuga menziesii* (Mirb.) Franco, a higher than 25% pruning severity decreased growth (Davel, 2013). It should also be considered that the evaluated periods could have been insufficient for *P. greggii* to show the positive effects of pruning on its growth since in a study on *Pinus nigra* Arnold subsp. pallasina, pruning improved growth in height and diameter up to 12 years after its application (Tonguc & Guner, 2017).

Fertilization is considered an essential silvicultural practice to increase biomass accumulation in forest plantation trees (Smethurst, 2010). Unlike the pruning effect, the results at La Soledad plantation corroborate the importance of trees fertilization to promote their growth (Calixto *et al.*, 2016). Particularly in *P. greggii*, an early fertilization trial confirmed this practice's importance for growth in low-productive areas (Vázquez-Cisneros *et al.*, 2018). The above coincides with the results of this research since the nutrient contribution favored the *P. greggii* plantation performance at La Soledad, which was established in a soil considered marginal due to its physical-chemical characteristics and its agricultural use history.

In other forest species, the nutrient supply in marginal soils has also been favorable to improve product performance. For example, in a *Eucalyptus urophylla* plantation with boron deficiencies, caused by edaphic acidity and high precipitation at the plantation site, the supply of this nutrient increased the tree's growth and volume (Rodríguez-Juárez *et al.*, 2014).

It is important to highlight the high growth values in trees without pruning due to fertilization. When comparing with pruned trees, it was found that fertilization stimulated growth in the non-pruned ones, because they conserved the nutrients and compounds related to the branches and foliage that are eliminated when pruning. Under natural conditions, foliage loss due to herbivory or another disturbing factor means important nutrient loss, which cannot be reabsorbed and retranslocated to other tissues for plant growth (Berendse *et al*., 2007; Turner, 2004). In *Cedrela odorata* L., foliage loss caused by the meliaceae borer (*Hypsipyla grandella* Zeller) generates a nutritional imbalance in the plants which affects their growth, an effect that is partially counteracted by fertilization (Calixto *et al*., 2015). This evidence suggests that fertilization in *P. greggii* offsets the negative effects of pruning on growth, especially in poorly productive areas. This result must be corroborated since during the evaluation period there was no interaction between the studied factors; therefore, it is recommended to examine in detail the interaction between pruning and fertilization over a longer period. These practices are often carried out simultaneously in forest plantations management and their joint effects are rarely examined (Forrester *et al*., 2012).

CONCLUSIONS

Pruning at 50 and 75% intensities in young high density plantations of *P. greggii* did not favor the growth of the trees in the evaluated study periods. On other hand, fertilization did promote this effect, therefore, it is advisable for it to be done in plantations established on marginal soils. It is recommended to examine other, lower than 50%, pruning intensities to explore in detail the effects of their interaction with fertilization, since the latter tends to offset the negative effects of pruning at high intensities.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Constructed wetlands as alternatives for swine sustainability

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ABSTRACT

Objective: To analyze available information about constructed wetlands and to identify the design, substrate, and vegetation advantages that could be an alternative solution to pig production system wastewaters.

Design/methodology/approach: A broad literature review was conducted to identify the main characteristics of constructed wetlands (CWs), as well as the various plant species associated with these systems, and the substrates used as filter beds.

Results: Vertical CWs have provided the best organic matter removal results, estimated indirectly through chemical oxygen demand (COD) and nitrogen derivatives such as total nitrogen (TN), ammonium (NH_3), nitrate $\left(NO_3^-\right)$, and nitrite $\left(NO_2^-\right)$. Several substrates are used as filter beds, but they must be evaluated according to their availability, cost, and feasibility for colonization by nitrifying and denitrifying bacteria to degrade the dissolved pollutants. Combinations of plant species can reduce more than 10% of nitrogen products and organic matter.

Study limitations/implications: The lack of monitoring for the satisfactory application of water care standards by small backyard and transition producers limits the adoption of environmental technologies for livestock sustainability in Mexico.

Findings/conclusions: Constructed wetlands are inexpensive, easy-to-use, adaptive systems that can be feasible alternatives for reducing the pollution caused by the swine wastewater generated by backyard producers.

Keywords: Wastewater, livestock pollution, wetland design, phytodepuration.

INTRODUCTION

Over the last decades, the nitrogen nutrients concentration in water tables has increased, resulting in a strong eutrophication problem, which limits the use of water for human consumption (Brix, 1997).

Image by Beatrice Hohl at Pixabay

Industrial, anthropogenic, and agricultural activities are the main sources of nitrogen. Agricultural activities include substantial amounts of excreta, urine, and uneaten food from pig farms that are discharged through wastewater (Pacheco and Cabrera, 2003).

Pig farm wastewater provides a 25,205-mg L^{-1} chemical oxygen demand (COD), 15,042-mg L^{-1} total soluble solids (TSS), 2,034-mg L^{-1} total nitrogen (TN), 2,032 mg L^{-1} total Kjeldahl nitrogen (TKN), 0.63-mg L^{-1} nitrous and nitric nitrogen $\left(N\text{-}\left(NO_2^- + NO_3^-\right)\right)$, and 1,760-mg L^{-1} ammonium $\left(N\text{-}NH_4^+\right)$. All of this depends on the number of animals, production process, local climate, among other factors (Garzón-Zúñiga and Buelna, 2014). While livestock activities contribute to rural development, it is necessary to perform them in a sustainable way, preventing pollutants from reaching natural waterbodies (Contreras-Contreras *et al.*, 2018).

Due to its size, backyard pig production is not usually considered as an activity with a significant contribution to nitrogen release; however, the establishment of small backyard farms in Mexican towns has boosted the risk of contamination of natural waterbodies. Small producers do not usually carry out water treatment; they indiscriminately discharge their wastewater —including solid waste— into waterbodies, croplands, or municipal drainage (Solís-Tejeda *et al.*, 2021). This causes soil contamination problems and unpleasant odors in the surrounding area.

In Mexico, the Law of National Waters (DOF, 2020) and the General Law of Ecological Balance and Environmental Protection (DOF, 2021) are implemented in a regulatory way. Those laws establish the following indicators: NOM-001-SEMARNAT-1996 that establishes the permissible pollutant limits for wastewater discharged in national waterbodies and assets; and the NOM-002-SEMARNAT-1996 that establishes the permissible pollutant limits for wastewater discharged in urban or municipal sewerage systems.

The waste released by livestock activities must be quantified in order to regulate it. Conventional wastewater treatment processes have high installation, infrastructure, maintenance, and labor costs; consequently, adopting them in production systems (such as backyard swine) is difficult. Therefore, it is important to provide small producers with affordable solutions that they can adopt and adapt (De la Mora *et al.*, 2014; Solís-Tejeda *et al.*, 2021).

A variety of technologies has been established worldwide to minimize the impact generated by livestock farms —particularly, pig farms. These technologies range from biodigesters or bioreactors (Venegas *et al.*, 2017) to practices of dietary management that minimize nitrogen release (Cervantes *et al.*, 2009).

In contrast, constructed wetlands (CWs) are a low cost, efficient, and easy-to-use sustainable technology that can be used to treat wastewater (from pig farms and other sources) with a high content of nitrogen derivatives (Sandoval-Herazo *et al.*, 2020). Consequently, the purpose of this literature review is to analyze the information generated about constructed wetlands and to identify the advantages of the designs, substrates, and vegetation that can be used as an alternative solution to swine production system wastewater.

Constructed Wetlands

The first constructed wetlands (CWs) —formerly known as "root zone method"— were developed by Seidel and Kickut in the 1970s (Brix, 1997). CWs are man-made engineering systems that —with the help of soils, vegetation, and microorganisms— are meant to mimic the processes of natural wetlands and are used to treat wastewater. The first CWs were used in the petrochemical industry, slaughterhouses, meat and dairy processing plants, and paper producing companies (Vymazal, 2014). These wetlands have efficiently reduced nitrogen and other environmental pollutants.

CWs reproduce natural processes and their effectiveness depends on the technical design specifications, type of substrate, hydraulic loading rates, plant species, flow type, hydraulic retention time, load of applied pollutants, among others (Jun *et al.*, 2017).

The substrate is the filter material to which bacteria that will degrade the compounds adhere; therefore, it must promote the development of microorganisms. The substrate adsorbs up to 90% of the pollutants (Luna and Ramírez, 2004).

The processes for the elimination or retention of nitrogen derivatives from the water in the CWs include: volatilization, nitrification, denitrification, nitrogen fixation by plants, microbial adsorption, mineralization (ammonification), nitrate reduction to ammonium (nitrate-ammonification), anaerobic ammonium oxidation (anammox), fragmentation, sorption, desorption, burial, and leaching. Microbial nitrification and denitrification are the most important of such processes (Jun *et al.*, 2017).

One or more methods for reduction of nitrogen derivatives can be managed according to the CW design. Nitrification is the use of nitrifying bacteria to oxidize ammonia (Jetten *et al.*, 1997). This process is achieved through the aeration of the problem water and by nitrifying bacteria. This usually takes places in the vertical flow CWs, where water cascades down through the substrate and dissolved oxygen is obtained (Sandoval-Herazo *et al.*, 2020). Denitrification (Figure 1) takes place under anaerobic conditions and is a process in which NO_3^- transforms into dinitrogen gas N_2 (Jetten *et al.*, 1997). This process takes place at the bottom of the wetlands where an anoxic environment is generated (Sandoval-Herazo *et al.*, 2020).

This establishes the theoretical basis for CWs as an alternative solution to high nitrogen wastewater discharges, in compliance with NOM-001-SEMARNAT-1996 and NOM-002-SEMARNAT-1996. In both cases, wastewater discharges must contain <40 mg L^{-1} (monthly sampling average) and 60 mg L^{-1} (daily average) of nitrogen.

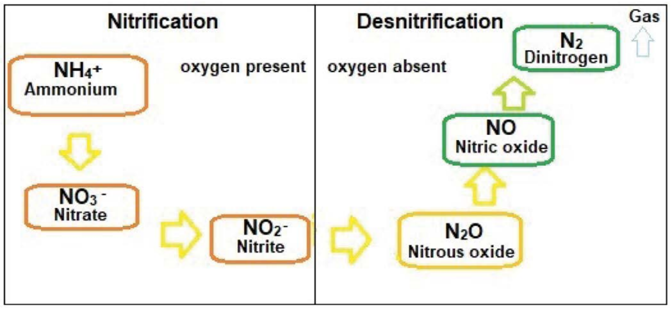


Figure 1. Nitrification and denitrification processes in constructed wetlands.

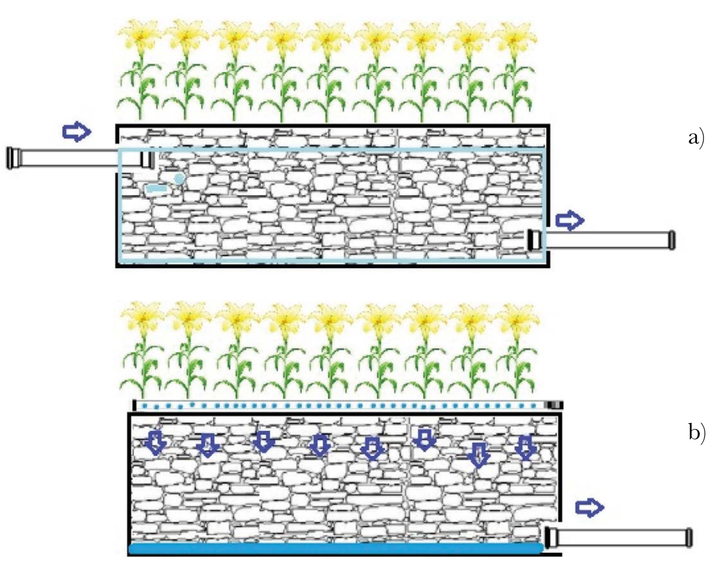
Constructed Wetland Design

Traditional CWs can be divided into two types: the surface CW (in which water flows over the substrate) and the subsurface CW (in which the water flows through the substrate). The latter can be categorized into two types: horizontal or vertical, according to the direction in which the water flows (Figure 2). There are hybrid systems made up of the union of two of these three designs. CWs with more than two stages —with the addition of mechanical or artificial aeration— have also been designed and lately circular flow designs are being evaluated (Rahman *et al.*, 2020).

Horizontal surface flow CWs have been assessed for swine wastewater treatment. To achieve an acceptable reduction of N and COD (>70%), a division into two blocks of treatment must be carried out, as a consequence of the high amount of nitrogen products (De la Mora *et al.*, 2014). Horizontal CWs are efficient secondary treatment systems which contributes to the elimination of the finest particles in the effluents and their installation requires a larger area (Jaramillo-Gallego *et al.*, 2016).

Vertical CWs have been used to treat wastewater with high N content, such as urban sewage. Treatment plants based on these systems have eliminated a high level of nitrogen pollutants (>90%) (Paing and Voisin, 2005). There are several ways to transform nitrogen through CW systems; however, only few processes can remove TN from wastewater. The removal of TN in both CWs varies between 40 and 55%, depending on the design and the input load. Nevertheless, the magnitude of the processes responsible for the removal of compounds differ between both systems. Vertical flow CWs successfully remove ammonia, but a poor denitrification takes place. Meanwhile, horizontal CWs provide good denitrification conditions; however, their capacity to nitrify ammonia is limited (Vymazal, 2007).

Vertical partially saturated CWs (VPS-CWs) combine nitrification with denitrification in a single system, maintaining an area with oxygen for nitrifying bacteria, as well as an



a) Horizontal constructed wetlands.

b) Vertical constructed wetlands.

Figure 2. Main constructed wetland designs.

anoxic area for the production of denitrifying bacteria. Compared with the treatment of pig wastewater with traditional vertical CWs, a higher reduction of pollutants —such as COD (5%), TSS (20%), N-NH4 (25%), TKN (32%), and CF (20%)— was found (Sandoval-Herazo *et al.*, 2020).

Substrates Used in Constructed Wetlands

Substrates are the main element of the CWs. They play an active role in water purification. Substrate porosity is a requisite for the facilitation of the pollutant adsorption process and it is a medium for the fixation of the bacterial biofilms that will transform the contaminants adsorbed by the material (Gao *et al.*, 2018). Substrate efficiency is affected by the hydraulic retention rate (HRT) which is the time that a volume of problem water remains in contact with the substrate (Jun *et al.*, 2017).

Based on these qualities, several types of substrates have been studied for the treatment of effluents from various activities (Table 1). It is important to consider the use of recycled, inexpensive, and readily-available materials to supply replacements (Zamora *et al.*, 2019).

Effect of Vegetation

The relationship between CWs and macrophytes has been studied since 1950 —particularly, emergent and submerged vegetation with floating leaves. All CWs efficiently remove organic matter and suspended solids; however, nitrogen removal may be lower, although this can be improved by vegetation (Vymazal, 2010). Plants must have certain characteristics to perform their function in CWs: 1) tolerance to high organic

Table 1. Main advantages and disadvantages of constructed wetlands.

Substrate	Advantages	Disadvantages	Author
Oyster shell	High reduction of total nitrogen (44.3%) and 73.1% of nitrite. Economic.	Requires very low HRR.	1
Zeolite	High reduction of total nitrogen (43%) and 22% of ammonia. Porous laminar structure. Facilitates chemical absorption and bacterial adhesion.	Costly.	1
Gypsum	Economic and easy to supply.	Low reduction of total nitrogen. Lower HRR compared to zeolite.	1
Ceramic	Reduces 45.8% of total nitrogen and 23.5% of ammonia. High microbial area.	Costly and difficult to supply.	1
Tezontle (coarse pumice)	Reduces 70% of COD and 49.2% of ammonia. Porosity 0.53. Large contact surface. Inexpensive and easy to supply.		2, 3
Sand (25%), peat (12.5%), pebbles (50%) and rock fragments (12.5%)	Reduces COD by 68% and ammonia by 66-83%. Inexpensive and easy to supply.	Low HRR to avoid clogging.	4
Porous construction stone (50%), 49% fine pumice (tepezil) and 1% soil	Reduces TSS by 34-35% and COD by 76-78%. Inexpensive and easy to supply.		5
Polyethylene terephthalate (50%), 49% fine pumice (tepezil) and 1% soil	Reduces TSS by 34-35% and COD by 76-78%. Very inexpensive, easy to supply, and ecological.		5

HRR=Hydraulic Retention Rate. COD=Chemical Oxygen Demand. TSS=Total Soluble Solids. 1= Jun *et al.*, 2017. 2=Mateo *et al.*, 2019. 3=Sandoval-Herazo *et al.*, 2020. 4=Rodríguez-González *et al.*, 2013. 5=Zamora *et al.*, 2019.

loads (5-day biochemical oxygen demand: BOD_5), between 3 and 25 g BOD_5 m^{-2} d; 2) abundant roots and rhizomes; and 3) above ground biomass to assimilate nutrients (Vymazal, 2011).

Plants degrade, absorb, and assimilate organic matter and nutrients (*e.g.*, nitrogen) in their tissues; furthermore, their roots provide a medium that favors bacterial growth and retain suspended solids, acting as a filter medium (Upadhyay *et al.*, 2016). Common plants —such as reeds (*Phragmites australis*), bulrushes (*Typha domingensis* Pers.) or tule (*Schoenoplectus* spp.)— have proved to be efficient and have therefore been studied. Recently, ornamental plants —such as gannet (*Zantedeschia aethiopica*) and lilies (*Canna* spp.)— have been researched. The performance of lilies in the absorption of pollutants improves the landscape; additionally, these plants can also be commercialized (Morales *et al.*, 2013).

The plant species that are intended to be used in the CWs must be previously studied: sometimes they do not have favorable effects and the organic matter of the roots of some of them may even increase values such as BOD_5 and COD (Jaramillo-Gallego *et al.*, 2016). When the system does not obtain the expected results, HRT can sometimes be adjusted, increasing the contact time with nitrifying and denitrifying bacteria.

Rodríguez-González *et al.* (2013) recommend yellow lily in vertical flow CWs to improve the removal of organic matter. Yellow lily improves the treatment for DOC elimination by 13%. In addition, it reduces 10% more ammoniacal nitrogen. De la Mora *et al.* (2014) tested surface flow CW related to bulrushes and species of the genus *Sirpus* to treat swine waters. Acceptable removal percentages attributed to the plants were achieved: 75% and 70% for COD and TN, respectively.

Yellow lily (*Iris pseudacorus*) improves the elimination of organic matter and nitrogen in CWs through plant adsorption by 13% (Rodríguez-González *et al.*, 2013). Sandoval-Herazo *et al.* (2020) compared the effect —reducing pollutants in dilute swine wastewater— of blue lilies (*Iris germanica*) as VPS-CW vegetation with lilies (*Canna hybrids*), removing >90% TN. No significant differences were observed between them. The adsorption effect of *Canna hybrids* was quantified by Mateo *et al.* (2019) who found an 18% reduction in $N\text{-}NO_4$.

CONCLUSIONS

The literature shows that constructed wetlands are efficient and low-cost systems for the treatment of wastewater with high loads of nitrogen products; therefore, they can be ideal for the treatment of swine wastewater. The horizontal constructed wetlands provide a considerable crop area for the exploitation of plants and reduce nitrogen; however, studies show that they have less impact than VPS-CWs or hybrid systems that combine nitrification and denitrification processes.

Further research supports that plants of the genus *Canna* absorb a large amount of nitrogen; these plants have suitable characteristics for the treatment of swine wastewater and they can also generate an additional income to production. Tests performed on substrates such as tezontle, tepezil, and recycled materials have efficiently eliminated nitrogen products and organic matter; they are also considered inexpensive and easy to acquire. In order to maximize its effectiveness, the size of the substrate particles and the hydraulic retention rate must be taken into account.

Government incentives focused on increasing livestock infrastructure must be directed towards the installation of swine production systems which consider constructed wetlands as an essential element in the reduction of environmental impacts.

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