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

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The Research Station Centro-Altos de Jalisco CIRPAC-INIFAP: celebration of its 50th anniversary

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National Research Institute for Forestry, Agriculture and Livestock (INIFAP by its acronym in Spanish)

INIFAP is a prestigious institution of scientific and technological excellence in México, recognized both nationally and internationally. It was established in 1985 from the merger of the National Institutes for Research in Forestry (INIF founded in 1958), Agriculture (INIA, founded in 1961), and Livestock (INIP founded in 1967), and its primary objectives have been to improve sustainable rural development, enhance competitiveness, and preserve the natural resources. INIFAP achieves these goals through collaborative work with other organizations, either public or private, as well as civil societies involved in the Mexican countryside activities. The institute focuses on generating scientific knowledge and technological innovations in the forestry, agricultural, and livestock sectors, responding to the needs of agro-industrial chains and various types of producers (INIFAP, 2024a).

INIFAP's mission is to develop technological solutions that drive innovation in the Mexican countryside. It envisions itself as a leading institution in creating technologies that benefit producer in the forestry, agricultural, and livestock sectors. The institute relies on 38 Research Stations pertaining to eight Regional Research Centers (CIR) distributed geographically throughout the country. In these locations, specialized researchers work to solve sector-specific and agro-ecological problems.

In addition to research stations, INIFAP manages six National Centers for Disciplinary Research (CENID), each known for a high level of specialization. These include: Water, Soil, Plant, and Atmosphere Relations (RASPA); Conservation and Improvement of Forest Ecosystems (COMEF); Animal Physiology and Improvement (FyMA); Animal Health

CIRPAC

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and Safety (SAI); Conservation of Genetic Resources (CNRG); and Family Agriculture (CENID AF) (INIFAP, 2024b).

Finally, INIFAP must align with the Convenio de Administración por Resultados 2020-2024. This agreement, following the current analysis, emphasizes the need for an agricultural policy model to address productive inequalities, promote research and technology development, and advance INIFAP's development program with its three main strategic themes: food security, sustainability of natural resources, and technological innovation.

Regional Research Center Pacífico Centro (CIRPAC by its acronym in Spanish)

CIRPAC-INIFAP covers four states in western México: Colima, Jalisco, Michoacán, and Nayarit. This region is known for its agro-ecological diversity, featuring climates that range from arid subtropical to very warm sub-humid tropical, and its outstanding forestry, agricultural, and livestock sectors. CIRPAC focuses on the region's key agrifood chains, which include crops such as corn, avocado, blue agave, sugar cane, vegetables, and cereals, as well as livestock and forestry, including pine, oak, and fir forests. Its mission is to generate and transfer technology and innovations to enhance the competitiveness and sustainability of the agricultural sector.

CIRPAC operates five Research Stations strategically located across its member states, along with two Experimental Sites, one in Jalisco and another in Nayarit. These facilities enable CIRPAC to achieve its objectives which are focused on addressing the region's research and technological development needs (INIFAP, 2024c).

Centro-Altos de Jalisco Research Station (CECEAJAL by its acronym in Spanish)

Altos de Jalisco Research Station was founded in 1974 to address the technological and information needs of agricultural producers in the region of Los Altos de Jalisco (Laborde, 1979), (Figure 1). Initially, research focused on crops such as corn, beans, sorghum, and the

Figure 1. The early days of the Altos Research Station.

corn-beans association. As the infrastructure expanded and more researchers joined, the scope of research widened to include sunflower, flaxseed, soybean, wheat, triticale, oats, and barley.

In 2003, an international evaluation underscored the importance of the Institute for Mexico's agricultural and forestry development. This recognition led to a restructuring that solidified Altos de Jalisco as a Strategic Research Station in the region. The station now covers the "Central" and "Altos" regions of Jalisco and collaborates with other institutions, such as the University of Guadalajara and various higher education centers, to develop research projects and facilitate technology transfer.

Currently, the Research Station is located in Tepatitlán de Morelos, Jalisco, covering most of the state, except for the North Coast and South Coast regions (Byerly, 2006). The facility spans 14 hectares, primarily dedicated to conduct research and extension. The scientific staff includes 30 researchers who work on forestry, agriculture, livestock, and cross-sectoral projects, as well as 10 administrative and support staff members (Figure 2). Forty percent of the researchers are recognized as part of the National System of Researchers from CONAHCYT, enhancing the impact and quality of the research conducted in CECEAJAL.

Forestry Research at CECEAJAL

México has a forest area of 138.7 million hectares, which provides various goods such as timber, food, medicines, and fibers, as well as essential ecosystem services like water and air purification, carbon sequestration, climate regulation, recreation, and landscape services (Torres-Rojo, Moreno-Sánchez, and Mendoza-Briseño, 2016). The state of Jalisco alone has a forest area of 4,850,337.4 hectares, encompassing diverse ecosystems and vegetation, including forests, jungles, mangroves, and arid and semi-arid zones (SEMARNAT, 2013). This diversity underscores the importance of conducting research to understand ecosystem functioning and develop tools to support decision-making by forest owners and managers.

Figure 2. Current staff at the Research Station Centro-Altos de Jalisco.

Currently, Centro-Altos de Jalisco Research Station is involved in three primary areas of forestry research: Sustainable Forest Management and Environmental Services, Plantations and Agroforestry Systems, and Forest Fires (Figure 3). Centro-Altos' research focuses on understanding ecosystem functioning to enhance the management of natural resources, including key timber species. Additionally, this station r has developed tools to better comprehend forest ecosystems and their responses to disturbances, such as fires. This includes studying aspects like forest fuels, fires behavior, fire risk and danger indicators.

In the Forestry Plantations research area, it has been evaluated the establishment and adaptation of various commercial forest species, including tropical species such as cedar, mahogany, parota, and bamboo. Additionally, studies have been conducted on the introduction, adaptation, and timber and latex production of exotic species like teak, melina, and rubber. Growth models for height and diameter, as well as mathematical models to estimate volume, biomass, and carbon for the most relevant taxa have also been developed (Figure 3).

Regarding environmental services, research has been focused on understanding and protecting biodiversity, regulating the hydrological cycle, controlling erosion, producing oxygen, and sequestering carbon, among other services. To advance knowledge on water production and quality, a unique forest basin was established in Tapalpa, Jalisco. This project has provided valuable information on the role of forests in maintaining minimal flow and erosion levels and has enabled the calibration of hydrological models for application to other watersheds. Furthermore, research has been conducted on non-timber products such as resin and oregano, which are integral to these forest ecosystems.

The Research Station has focused on developing, implementing, and transferring new knowledge in forestry, impacting national, regional, and local levels. A key project, the System of Integrated Management of Forest Resources (SIMANIN), was developed for the Sierra de Tapalpa forests and later applied to other regions. SIMANIN, based on a 50-year intensive management plan with five cutting cycles, was designed using producer experience and research from Permanent Silvicultural Research Plots (SPIS) established in the 1980s. The project, launched in 1992, covers 8,000 hectares and includes commercial thinning, regeneration methods with seed trees, and the release of newly established cohorts (Manzanilla *et al.*, 1997).

Figure 3. Research areas in forest sciences at the Research Station.

Another significant project is the National Forest and Soil Inventory (INFyS), which began in 2003 and was developed for the National Forestry Commission (CONAFOR) under the Ministry of Environment and Natural Resources (SEMARNAT). This project established the methodological foundations, sampling design, sample size, and the structure and size of sampling sites, as well as the analysis scheme for obtaining dasometric parameters.

In 2004, SEMARNAT, CONAFOR, the National Institute of Statistics and Geography (INEGI), the National Electoral Institute (INE), and INIFAP formalized the structure of the National Forest and Soil Inventory project and outlined general guidelines in a guiding document. INIFAP was responsible for collecting field data in various states across the country. This data was then statistically processed to generate results and compile state-specific reports.

Currently, several national and regional projects are underway. One notable project is the "Adaptation, Generation, and Integration of Technologies for Sustainable Forest Management in Jalisco and Michoacán". This initiative aims to validate and calibrate growth models for the region and commercially relevant forest species, and integrate these models into user-friendly software, such as ForestSimulator and Shiny applications in Java and R-JavaScript languages, respectively (Figure 4). This will facilitate their use in the forestry sector. Additionally, we are gaining experience in environmental restoration through projects such as identifying priority areas for implementing restoration strategies in ecosystems affected by forest fires. This project will develop methodologies to define priority areas and restoration strategies.

Looking ahead, forestry research at CECEAJAL faces important challenges and opportunities. Climate change, agricultural expansion, and urbanization continue to exert pressure on forests and their biodiversity. Therefore, it is crucial to intensify research in areas such as ecological forestry, precision forestry, ecological restoration, climate change adaptation, and the valuation of ecosystem services.

The incorporation of emerging technologies, such as forest growth simulators, geographic information systems (GIS), and satellite monitoring, presents new opportunities for enhancing forest management and land-use planning. These tools will enable more effective resource management by providing accurate and up-to-date data to support decision-making.

Figure 4. New forestry technologies at the research station.

Currently, there are two laboratories at national level, the forest fire laboratory and another dedicated to integrating emerging technological components for the use, conservation, and restoration of forest ecosystems. However, it is crucial to equip these laboratories with state-of-the-art technology to ensure that the institute remains a leader in research and sustainable forest management at both national and regional levels.

Agricultural Research at CECEAJAL

In the agricultural sector, new varieties of major crops have been developed for the region, along with management recommendations for fertilization, pest control, disease management, and weed control. These efforts aim to enhance or maintain the yield and productivity of local producers. Research focuses on crops including corn, beans, small-grain cereals (such as oats, wheat, barley, and triticale), chickpeas, agave, and forage crops (such as forage corn and pasture), as well as oilseeds (including canola, safflower, soybeans, and amaranth) (CIRPAC, 2008).

The Research Station currently supports agricultural research programs for corn, wheat, oats, and vegetables. Through breeding programs, researchers have continuously developed and promoted new varieties of corn, beans, wheat, oats, barley, and chickpeas. This progress is facilitated by INIFAP's collaboration with national companies and producer associations that focus on the production of certified seeds. These seeds are supplied in a timely manner, meeting demand at accessible prices, and extending their availability to the regional level or the Central Pacific area of influence.

The Research Station is also engaged in research on agave, hibiscus, chia, oilseeds, and small-grain cereals (Figure 5). For agave, research includes integrated management of phytosanitary issues, genetic improvement, and agronomic practices. For hibiscus, studies focus on functional compound content and agricultural mechanization. Oilseeds research (canola, safflower, and soybean) primarily targets agronomic management. In the case of basic grains (wheat, barley, and oats), the research emphasizes agronomic management, genetic improvement, and integrated management of phytosanitary problems.

Since 1979, there has been a genetic improvement program for corn varieties aimed at developing hybrids and improved varieties of white and yellow corn (Ramírez *et al.*, 1996). The program's goal is to produce certified seed for companies and producer groups, targeting grain for the dough and tortilla industry, balanced food, and forage (both fresh

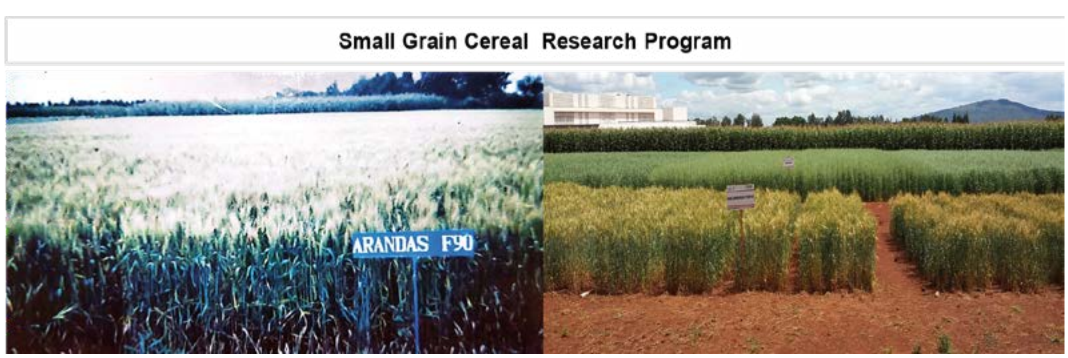

Figure 5. Evaluation and transfer of technology in small grain cereals.

and dry). This initiative has had a nationwide impact, particularly in regions similar to the Central Pacific. Over the past 15 years, CECEAJAL has made over 10 hybrids of white and yellow corn available to producers and seed companies in response to their demands (Figure 6).

The corn research program also includes agronomic management, seed production technology, training for producers and technicians on certified seed production, integrated management of phytosanitary problems, and support for technology transfer. Looking ahead, the Research Station aims to advance towards the agriculture of the future. This vision encompasses the production of food, goods, and ecosystem services that are compatible with natural resource conservation and the use of field by-products as bio-inputs. It also involves generating varieties resilient to climate change, grounded in scientific knowledge, technology, and innovation, while considering national and international trends and developing strategies for their validation and implementation.

Livestock Research at CECEAJAL

The state of Jalisco is recognized as a leading producer of animal protein in México, with notable contributions in bovine milk and meat, pork meat, eggs and poultry meat (Panorama Agroalimentario, 2024). Additionally, Jalisco produces significant amounts of small ruminant meat and honey (Panorama Agroalimentario, 2024). These high production levels reflect the strong commitment of Jalisco's society to primary sector activities (Rostros de Jalisco, 2016). Consequently, animal production systems hold a crucial role in the state from social, economic, and environmental perspectives (Cervantes *et al.*, 2001).

INIFAP has historically engaged in research programs focused on the above mentioned species. Research, training, and technology transfer activities are tailored to the specific socioeconomic, technological, and environmental characteristics of each production system (Programa de Desarrollo del INIFAP, 2018). For instance, in Mexico, bovine milk is produced through various systems: the intensive system predominant in states like Coahuila and Durango, the family or semi-intensive system predominant in Jalisco and Estado de México, and the dual-purpose system found in Veracruz and Chiapas (PNI, 2023).

Figure 6. Technology available to producers dedicated to the production of corn seed.

Livestock activities are approached from a process perspective, where products (meat or milk) are transformed from inputs such as fodder and animals through activities linked to a production unit (Núñez *et al.*, 2009). This process approach involves the identification and measurement of indicators generated in each sub-process, including forage production, feeding, reproduction, health, genetics, replacement breeding, and management. This methodology allows the evaluation of whether objectives are being met and tracking progress over time, which is essential for decision-making.

At the Centro-Altos de Jalisco Research Station, the primary research programs focus on dairy cattle managed under the family or semi-intensive milk- production system, beef cattle in the cow-calf system, and swine production. Although the Research Station does not have specialists for every issue within these production systems, it collaborates with specialists from other INIFAP centers across the country to address these challenges. The animal reproduction area constitutes a strength for the livestock sector in this station, since the recent establishment of a laboratory and the integration of researchers with a high specialization in reproductive biology, assisted reproductive technologies, and applied reproductive management (Figure 7). Other strengths include the presence of a module for integrated management of farm waste and swine production (Figure 8) as well as researchers with specialization in forage management and animal nutrition who have extensive experience and tight relations with animal production units and producer organizations.

In terms of research activities, the family milk-production system has seen projects aimed at identifying issues and developing strategies to address them, particularly in forage management, feeding, reproduction, genetics, replacement heifers rearing, and health. For the cow-calf production system, research has primarily targeted feeding and reproduction processes to enhance reproductive and productive performance. In swine production, projects have concentrated on managing farm waste and improving swine health and production (Figure 8).

The knowledge generated from these projects is disseminated through scientific articles and institutional brochures written in accessible language for producers and the general public. Additionally, this knowledge is utilized in courses and workshops designed to train

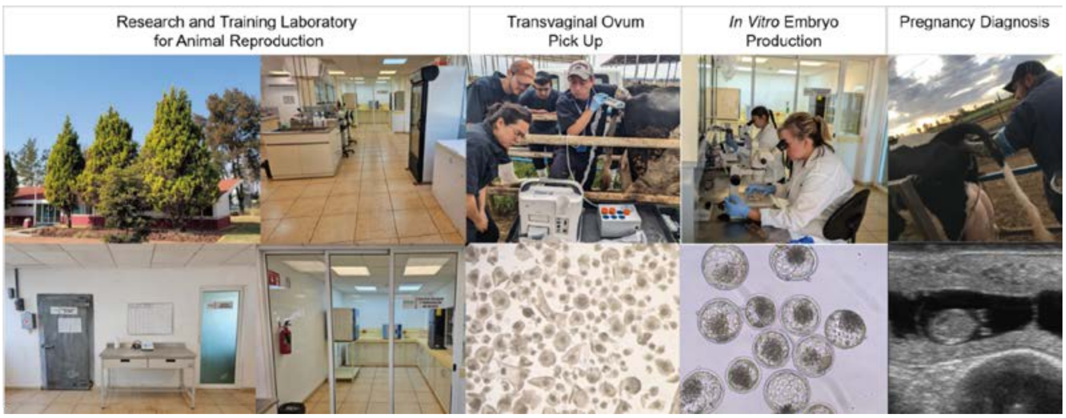

Figure 7. Research and training Laboratory for Animal Reproduction at the Centro-Altos Research Station.

Figure 8. Research and training Module in Managing Farm Waste and Swine Production at the Centro-Altos Research Station.

students, producers, and professionals working in either private sector or government programs related to these animal production systems (Figure 9).

While current projects are aimed to improve the productivity, competitiveness, and sustainability of production systems, there is still progress needed to fully address the research needs of the livestock sector. Environmental changes, such as erratic rainfall patterns, temperature fluctuations, and droughts resulting from climate change, call for the development of strategies that enhance system resilience (Godde *et al.*, 2021). Efforts must also focus on reducing methane emissions and the water footprint per kilogram of protein (milk, meat, or eggs) per animal unit. Improving each sub-process within production units can contribute to these goals.

INIFAP envisions that the adoption of technologies such as automation, artificial intelligence, assisted reproductive technologies, and genomic selection, among others will significantly improve animal production and sustainability. These advancements are essential for meeting the growing global food demands and addressing the challenges posed by increasingly adverse climate scenarios (Agricultura del Futuro, 2024).

Figure 9. Training activities in Beef and Dairy Cattle Production Systems.

Final Remarks

Over its 50 years, INIFAP's Centro-Altos de Jalisco Research Station has been pivotal in advancing forestry, agricultural, and livestock research and development in Jalisco as well as neighboring regions. Its ability to adapt to the evolving challenges and demands of the agricultural sector has established this Research Station as a cornerstone of knowledge generation and technology transfer.

The consolidation of the Research Station as a strategic institution has not only enhanced its infrastructure and human resources but has also broadened its influence. This expansion has significantly impacted sustainable rural development and the competitiveness of the agricultural sector. Strategic partnerships with educational institutions and other organizations have been crucial in expanding knowledge and training new generations of agricultural professionals.

Looking forward, the future of the Centro-Altos de Jalisco Research Station is closely tied to its capacity for innovation and adaptation to emerging realities, such as climate change and the need for more sustainable agricultural practices. Integrating new technologies and scientific methods into its research and development programs will be essential for continuing to address the challenges facing Mexican agriculture effectively.

In summary, the Centro-Altos de Jalisco Research Station has not only observed but actively contributed to the evolution of the agricultural sector over the past five decades. Its achievements reflect the dedication and vision of its researchers and collaborators, who have worked relentlessly to enhance the lives of producers and the sustainability of natural resources. The ongoing challenge will be to sustain leadership in innovation and technology transfer, ensuring that future generations benefit from a thriving and resilient agricultural sector.

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Colegio de Postgraduados

Genotype by Environment Interaction of Maize (*Zea mays* L.) Hybrid Yield in Guanajuato, Mexico

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ABSTRACT
Objective: To evaluate phenological and yield parameters in experimental maize (*Zea mays* L.) hybrids across different environments.
Design/Methodology/Approach: The trials were conducted under gravity irrigation conditions with 21 experimental maize crosses and four commercial hybrids. The experiment was established in three communities in Guanajuato, Mexico, during the spring-summer agricultural cycle. A randomized complete block design with three replications was used in each environment. Genotype by environment interaction analysis was performed using the AMMI model.
Results: Genotypes 23, 21, and 16 achieved the highest yield, followed by genotypes 22, 6, 17, and 5, while genotypes 8 and 13 showed the lowest yield.
Limitations of the Study/Implications: The promotion of these hybrids in environments within the state of Guanajuato is desirable.
Findings/Conclusions: The genotypes exhibited high genetic divergence in the expression of yield parameters and their components. The outstanding hybrids were 23, 21, and 16, showing higher yields across all locations and demonstrating better adaptation to the three evaluation environments.

Keywords: Hybrids, Stability, Maize, Yield.

INTRODUCTION

The primary form of maize (*Zea mays* L.) consumption in Mexico is the tortilla, which is why it holds the top position in the basic food basket for the society. Mexico ranks seventh globally in maize production, with a total of 27.5 million tons produced in 2021 (FAOSTAT,

2023). In Mexico, maize cultivation is the leading crop, with six million hectares planted, followed by bean (*Phaseolus vulgaris* L.) production with one million hectares, and sorghum in third place with 1.367 million hectares (SIAP, 2022). In 2022, Guanajuato produced 1,734,381 tons of maize for grain with an average yield of 5.40 t ha^{-1} (SIAP, 2023). The state's main agricultural activity occurs during the spring-summer agricultural cycle, accounting for 75.5% of the cultivated area (SIAP, 2018). Given the importance of this crop, it is essential to implement strategies to provide the agricultural sector and society with viable alternatives for the use of elite maize materials that have good yield potential and are adaptable to diverse environmental factors.

An efficient option is the use of hybrids developed through the process of genetic improvement. In this context, the main objective of genetic breeding programs is to obtain genotypes with higher yields; however, in most cases, yield potential is masked by genotype by environment interaction (G×E). This occurs when genotypes respond differently to environmental variations (Gordón-Mendoza *et al.*, 2006). The genotype by environment interaction (G×E) model has been crucial in identifying the productive potential of varieties and hybrids in different crops (Williams *et al.*, 2021). Sprague and Eberhart (1977) mention that unpredictable environmental factors exist, which is why it is advisable to increase the number of environments for the evaluation of genetic materials. New multivariate methodologies not only allow for the description of genotype by environment interaction but also provide deeper insights into the nature of this interaction. Among these methodologies, the Additive Main Effects and Multiplicative Interaction (AMMI) model stands out for its ability to interpret many genotypes across various environments (Crossa *et al.*, 1990). This method is currently one of the most widely used for interpreting stability in maize (Ledesma *et al.*, 2012), wheat (Vázquez *et al.*, 2012), and sorghum (Williams *et al.*, 2021). In maize cultivation, this model has proven its efficiency in identifying outstanding and stable materials for different ecological niches (González *et al.*, 2009; Torres *et al.*, 2011; López *et al.*, 2019; Lopez *et al.*, 2017). There is evidence supporting the efficient use of the AMMI model for identifying genotypes in different locations; therefore, phenological and yield parameters were evaluated in outstanding experimental maize hybrids across three environments.

MATERIALS AND METHODS

Location and Genetic Material

The trials were conducted under gravity irrigation conditions with 21 experimental maize (*Zea mays* L.) crosses and four commercial hybrids (Puma, Cimarrón, DK-2061, and San Andrés). The evaluation was carried out in the communities of Soria in the municipality of Comonfort, Empalme Escobedo, and Juventino Rosas, in the state of Guanajuato, Mexico, during the spring-summer agricultural cycle of 2015.

Experimental Design and Agronomic Management

The experimental plot for each treatment consisted of two rows, each 5.2 meters long, with 0.76 meters between rows and 14 cm between plants. At planting, a chemical formula of 120N-80P-60K was applied, and during the second weeding, 120N-00P-00K was applied. A randomized complete block design with three replications was used in each environment. Agronomic management followed the technological package of INIFAP for irrigation conditions in the region (INIFAP, 2015).

Evaluated Variables

The following variables were evaluated: days to male flowering (DMF), plant height (PH), ear height (EH), rust (R) (*Puccinia sorghi*), and incidence and severity of Exserohilum (HLM). For the assessment of incidence and severity, the scale proposed by Arrieta *et al.* (2007) was used, which classifies severity on a scale from 1 to 9, where: 1=no disease (0%), 2=minimal presence of disease (1-10%), 3=light infection (11-20%), 4= value between light and moderate (21-34%), 5=moderate infection (35-49%), 6=value between moderate and severe (50-64%), 7=severe infection (65-78%), 8=value between severe and very severe (79-89%), and 9=very severe infection (>90%).

The number of plants affected by stem rot caused by *Fusarium moniliforme* (FUS) was quantified, as well as ear coverage (EC) using a scale from 1 to 5, where: 1=excellent coverage (100% of the population with covered ears), 2=fair coverage (75-99% of the population with covered ears), 3=exposed tip (50-74% of the population with covered ears), 4=exposed grain (25-49% of the population with covered ears), and 5=completely unacceptable (>25% of the population with covered ears).

Four ears were harvested to estimate post-harvest variables. The following measurements were taken weight of 500 grains (W500G), weight of grain per ear (WGE), number of rows (ROW), grain yield in tons per hectare adjusted to 14% moisture (YIELD), grains per ear (G×E), grains per row (G×ROW), ear perimeter (PER), and ear length (EL).

A combined analysis of variance (ANOVA) was performed for the main effects of genotype (G) and environment (E) using the following model:

$$Y_{ijk} = \mu + G_i + A_j + (GA)_{ij} + Bk\left(A_j\right) + E_{ijk}$$

where: Y_{ijk}=average yield of the *i*-th genotype obtained in the *j*-th environment and *k*-th replication, μ=overall mean effect, G_i=effect of the *i*-th genotype, Aj=effect of the *j*-th environment, $(GA)_{ij}$= interaction effect between the *i*-th genotype and the *j*-th environment, $Bk\left(A_j\right)$=effect of the *k*-th replication in the *j*-th environment, E_{ijk}=random error effect associated with the *i*-th genotype in the *j*-th environment and *k*-th replication.

A Principal Components Analysis (PCA) was also conducted to evaluate the non-additive effects of the G×E interaction (Gollob, 1968). This model, known as AMMI (Additive Main Effects and Multiplicative Interaction), developed by Gauch and Zobel (1988), includes both additive and multiplicative parameters. The data were analyzed using the SAS statistical package (SAS, 2006). Mean comparisons for agronomic traits were performed using Tukey's test ($p \leq 0.05$). The analysis of genotype by environment interaction using the AMMI model was carried out with the R software (R Core Team, 2012).

RESULTS AND DISCUSSION

The principal components analysis related to the eigenvalues of the correlation matrix showed that the first two components accounted for 51.6% of the variation with respect to the evaluated variables (Figure 1). Principal Component 1 (PC1) explained 36% and PC2 explained 15.6%. These values are considered acceptable for representing reliability with respect to the total variance relationships of the parameters under study (Arroyo *et al.*, 2005).

PC1 showed a positive association with the variables EL, G×ROW, EH, PH, G×E2, WGE2, YIELD, and DMF (Figure 2a), while PC2 recorded a positive association with the variables R and PL and a negative association with the variables ROW and PER (Figure 2b).

The color measurement indicates the percentage contribution of the evaluated variables (PC1 and PC2), where colors closer to red represent higher percentages of contribution, while colors closer to blue indicate lower percentages. The variables of interest are those with shades closest to red and higher percentages (Figure 3).

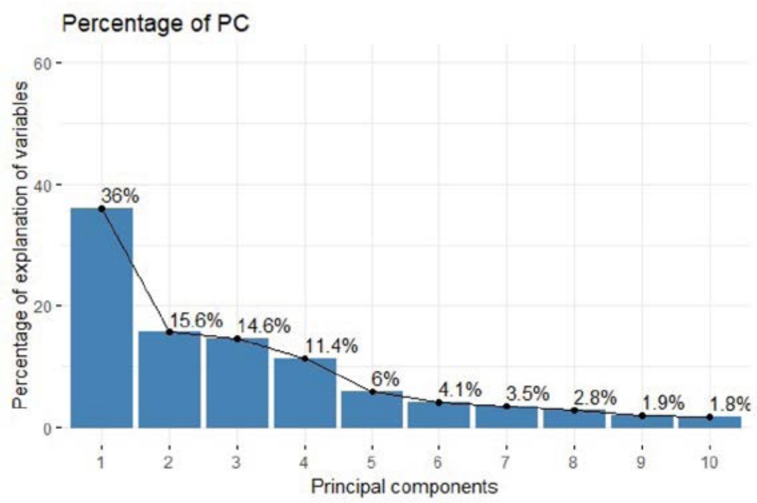

Figure 1. Percentage contribution of the principal components.

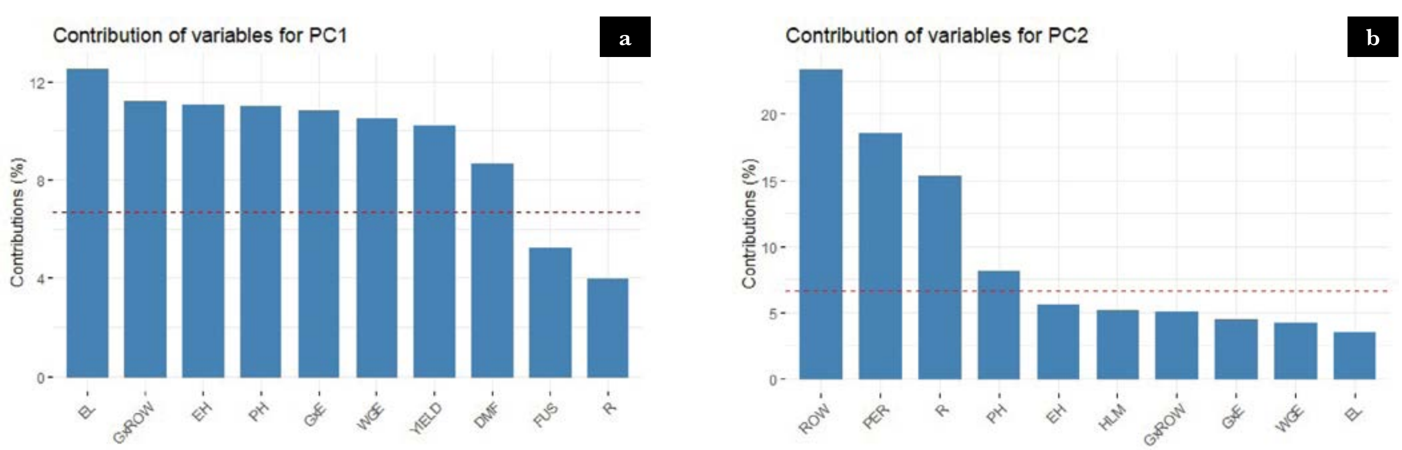

Figure 2. a) Percentage contribution explained by PC1. b) PC2 in relation to the study variables.

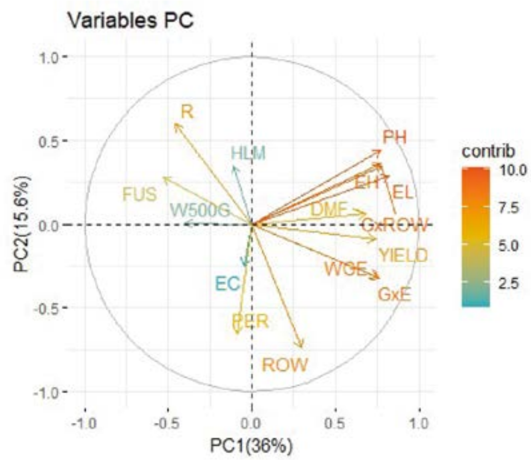

Figure 3. Biplot of agronomic variables evaluated in 25 maize hybrids across three locations in Guanajuato, Mexico.

The angle of the vectors explains the association between variables, such that smaller angles between vectors indicate a strong association, while larger angles indicate no association. Therefore, a strong relationship was observed among the variables PH, EH, G×ROW, EL, DMF, YIELD, WGE, and G×E, as well as between PER and ROW, R, and FUS. A low relationship was observed between FUS and G×E and GWE.

Analysis of variance across environments

The analysis of variance across locations (Table 1) detected highly significant differences (P≤0.01) for the sources of variation among locations and treatments concerning all the studied traits. Regarding the interaction between locations and treatments, highly significant differences (P≤0.01) were found for the variables DMF, PH, EH, HLM, FUS, R, EC, W500G, and YIELD, indicating an interaction between locations concerning the treatments. Concerning repetitions within locations, there were differences (P≤0.01) in DMF, PH, EH, EC, WGE, and YIELD, but discrepancies (P≤0.05) in G×E. The coefficients of variation ranged from 1.3% to 24.0%, which are acceptable values for ensuring the study's reproducibility over time and space. Therefore, these data demonstrate the reliability of the findings throughout this research.

Mean comparisons for locations showed that the shortest days to male flowering (DMF) were recorded in Soria with 71 days, while the longest were in Juventino Rosas with 75 days (Table 2). Extreme values for plant height (PH) and ear height (EH) were observed between Comonfort (243 for PH and 130 for EH) and Juventino Rosas (198 for PH and 103 for EH). In terms of Helminthosporium (HLM), the location with the highest damage to genotypes was Soria (3.2%), while the lowest presence was in Juventino Rosas (3%). These values are low according to the scale proposed by Arrieta *et al.* (2007), indicating that the evaluated hybrids are tolerant to HLM, showing only a slight infection of this pathogen. Regarding Fusarium (FUS), the location with the highest damage was Comonfort with 7.4%, while the lowest incidence was observed in Juventino Rosas with 3.8%. On the other hand, for the variable of rust (R), the location with the most damage

Table 1. Mean squares across locations for variables in three locations of the experiment.

S.V.	D.F	DMF	PH	EH	HLM	FUS	R	EC	W500G	WGE	ROW	YIELD	G×E	G×ROW	PER	EL
Loc	2	313.2**	41823.3**	13761.6**	7.0**	243.3**	21.1**	1.9**	10921.0**	224056.0**	13.4**	154.6**	29582.4**	93.3**	9.0**	132.4**
Rep(Loc)	6	3.3**	423.3**	329.6**	0.4	0.6	0.5	1.2**	368.0	26502.9**	3.0	3.8**	8180.0*	14.5	1.1	8.8
Treat	24	38.5**	1190.1**	1111.6**	2.6**	34.6**	3.9**	4.0**	2091.6**	16158.6**	12.6**	22.7**	29442.7**	60.7**	2.2**	5.4**
Loc×Treat	48	9.8**	220.9**	248.5**	0.9**	25.1**	1.0**	1.0**	557.1**	6790.2	2.3	3.5**	4290.7	11.6	0.7	2.1
Error	144	1.0	68.9	63.3	0.3	0.3	0.2	0.2	201.2	5923.5	1.6	0.8	3211.3	9.5	0.7	1.6
C.V. (%)		1.3	3.7	6.7	19.2	11.4	19.9	24.0	7.9	14.2	8.3	7.0	10.5	8.9	5.1	8.2
Media		73.6	223.9	117.3	2.9	5.4	2.6	2.1	179.3	540.1	15.5	13.1	536.0	34.5	16.5	15.7

S.V.=Source of variation; DF=Degrees of Freedom, DMF=Days of Male Floration; PH=Plant Height; EH=Ear Height; HLM=helmitosporium; FUS=fusarium; R=Rust; EC=Ear coverage; W500G=Weight of 500 grains; WGE=Weight of grain per ear; ROW=Number of rows; YIELD=Grain Yield tons per hectare; G×E=Grains per Ear; G×ROW=Grains per Row; PER=Ear Perimeter; EL=Ear length; Loc=Locations; Rep(Loc): replicates by location; Treat=Treatment; Loc×Treat=Location by treatment; C.V.=Coefficient of Variation. *Significance levels: *, **=different at $P \leq 0.05$ and 0.01, respectively.

Table 2. Mean comparisons by location for the traits measured in the experiment.

Location	DMF	PH	EH	HLM	FUS	R	EC	W500G	GWE	ROW	YIELD	G×E	G×ROW	PER	EL
Comonfort	74.42 b	243.26 a	130.44 a	2.64 c	7.42 a	2.48 b	2.30 a	187.12 a	592.45 a	15.78 a	14.37 a	558.96 a	35.48 a	16.46 b	17.30 a
Juventino Rosas	75.13 a	198.65 c	103.38 c	2.93 b	3.88 c	2.20 c	1.99 b	165.48 b	483.40 c	15.06 b	11.57 c	523.65 b	35.77 a	16.19 b	14.88 b
Soria	71.29 c	231.06 b	118.16 b	3.25 a	5.10 b	3.22 a	2.12 ab	185.55 a	544.47 b	15. 81 a	13.54 b	525.54 b	33.29 b	16.88 a	15.13 b
DHS	0.39	3.21	3.07	0.21	0.24	0.20	0.19	5.48	29.76	0.49	0.35	21.90	1.19	0.32	0.50

DMF=Days to male flowering; PH=Plant height; EH=Ear height; HLM=Helminthosporium; FUS=Fusarium; R=Rust; EC=Ear Coverage; W500G=Weight of 500 grains; GWE=Grain weight per ear; ROW=Number of rows; YIELD=Grain yield in tons per hectare; G×E=Grains per ear; G×ROW=Grains per row; PER=Ear Perimeter; EL=Ear length; HSD=Honest significant difference.

was Soria, and the least damage was in Juventino Rosas. For ear coverage, the location with the highest coverage in genotypes was Comonfort (2.3%), while the lowest coverage was in Juventino Rosas (2%). This indicates that the materials had regular coverage relative to the total population, which is a very important characteristic for obtaining high-quality grain by preventing the ear from being exposed to physical damage from pests and diseases. For the weight of 500 grains (W500G) and number of rows (ROW), Comonfort and Soria were the locations with the highest W500G and ROW, while Juventino Rosas exhibited lower W500G and ROW.

Regarding maize ear weight (WGE), the location with the highest ear weight was Comonfort, while the lowest ear weight was observed in Juventino Rosas. Concerning grain yield (YIELD), the genotypes showed the highest yield in Comonfort with 14 t ha^{-1}, and the lowest yield in Juventino Rosas with 11 t ha^{-1}. There was a difference of 3 t ha^{-1} between the locations with the highest and lowest grain yield. For grains per ear (G×E) and ear length (EL), Comonfort was found to have the highest G×E and EL, while Juventino Rosas and Soria had lower values. In terms of grains per row (G×ROW), Comonfort and Juventino Rosas had the highest values, with Soria showing the lowest. However, for ear perimeter (PER), Soria had the largest perimeter, while Comonfort and Juventino Rosas had the smallest. It is noteworthy that in Comonfort, the genotypes expressed their highest genetic potential in most of the studied variables, which is an important factor for achieving high grain yield.

In Table 3, the mean comparisons of the studied variables across the three evaluation environments are presented. It was observed that nine hybrids were statistically superior to the average yield of the check varieties, which were hybrids 23, 21, 16, 20, 22, 6, 17, 5, and 2, with yield increases of 17.2%, 16.6%, 16.5%, 15.8%, 8.6%, 7.7%, 7.5%, 5.6%, and 0.75%, respectively. These results demonstrate that there are experimental hybrids with similar or better performance compared to the genotypes used as checks, as seven hybrids from the selected group based on the average yield of the checks showed superior yields of over 14 t ha^{-1}. Regarding the DMF variable, the earliest hybrid was number 11 with 70 days, while the latest-maturing hybrid was number 25 with 77 days. On the other hand, the hybrids with the greatest plant height were 17 (241 cm) and 25 (239 cm), while the shortest was hybrid 7 with 200 cm. The hybrid with the greatest ear height was 22 with 146 cm, and the lowest was hybrid 7 with 100 cm. For the HLM variable, the hybrid with the highest percentage was 14 with a rating of 4.00, and the lowest was hybrid 2 with 1.6% damage. Regarding cob coverage, the hybrid with the highest rating was 14 with 3.8%, while the lowest was hybrid 4 with a rating of 1.2%. For the Fusarium variable, the hybrid with the most damage was 13 with 11%, and the least was 21 with 2.5%. In the rust variable, the hybrid with the highest rating was 13 with 3.8% damage, while hybrids 4 and 1 showed the least damage, both with a rating of 1.4%. The hybrid with the highest 500-grain weight was 10 with 216.8 g, while the hybrid with the lowest weight was 17 with 154 g. Regarding WGE, the hybrids behaved similarly, with a range from 428.7 to 600.4, corresponding to genotypes 13 and 16. Materials 6 and 8 showed the highest number of rows with 17, followed by material 14 with 17; however, the hybrid with the fewest rows was 13 with 12 rows. For the yield variable, hybrids 23, 21, and 16 had the highest yields

Table 3. Mean Comparisons of the Traits Measured in the Experiment Evaluated in the Three Locations in Mexico.

TREAT	DMF	PH	EH	HLM	FUS	R	EC	W500G	GWE	ROW	YIELD	G×E	G×ROW	PER	EL	%/CON
1(T)	75.33 bcd	214.33 g-k	113.00 e-i	1.88 fg	7.33 c	1.44 h	2.44 c-f	174.22 c-h	547.00 ab	16.44 ab	12.95 c-h	565.11 a-f	34.44 b-e	16.84 abc	15.38 abc	−3.34
2(T)	73.66 def	221.55 d-h	105.11 hi	1.66 g	6.77 cd	1.66 fgh	2.33 d-g	193.77 a-d	561.44 ab	15.55 a-d	13.94 b-e	503.33 d-h	32.44 c-f	16.44 a-d	15.72 abc	4.05
3(T)	74.33 cde	220.88 e-h	107.11 hi	2.33 d-g	4.66 ghi	2.22 d-h	1.55 f-i	170.11 d-h	554.56 ab	16.44 ab	13.43 c-g	569.56 a-f	34.77 b-e	16.56 a-d	15.00 abc	0.26
4(T)	76.00 abc	230.55 a-f	123.55 b-g	2.00 efg	4.22 ghi	1.44 h	1.22 i	168.11 e-h	520.56 ab	16.00 abc	13.29 c-g	548.89 b-f	34.33 b-e	16.26 a-d	16.11 abc	−0.81
5	73.77 def	229.66 a-f	123.22 b-g	2.88 cde	5.11 efg	2.22 d-h	3.55 ab	17.94 c-h	575.11 a	16.66 ab	14.15 a-d	597.33 a-d	35.88 a-e	16.32 a-d	16.66 ab	5.63
6	72.44 fgh	234.66 a-e	125.22 b-f	3.00 bcd	4.55 ghi	2.44 c-g	1.66 e-i	172 d-h	595.56 a	17.33 a	14.43 a-d	630.67 ab	36.44 a-e	17.20 ab	16.50 ab	7.74
7	72.66 efg	200.11 k	100.88 i	3.88 ab	9.66 b	3.22 abc	2.22 d-h	177.00 c-h	504.22 ab	15.55 a-d	11.62 hij	492.00 e-h	31.77 ef	16.61 a-d	14.38 bc	−13.23
8	71.11 ghi	203.77 kj	107.66 hi	3.33 abc	5.77 def	3.44 ab	2.77 bcd	193.77 a-d	487.78 ab	17.33 a	10.29 j	495.33 e-h	28.44 f	17.14 ab	14.00 c	−23.21
9	71.77 ghi	224.44 b-h	110.66 ghi	3.22 a-d	5.77 def	2.88 b-e	2.11 d-i	184.61 b-f	509.22 ab	16.22 ab	12.38 e-h	504.89 d-h	31.44 ef	16.74 a-d	15.50 abc	−7.61
10	70.88 hi	205.44 ijk	107.11 hi	3.11 a-d	6.33 dc	2.77 b-e	2.11 d-i	216.88 a	494.33 ab	13.33 ed	11.41 hij	415.78 h	31.22 ef	16.33 a-d	14.70 abc	−14.79
11	70.44 hi	212.77 h-k	111.88 ghi	2.88 cde	5.77 def	2.55 b-f	1.88 d-i	169.77 d-h	523.67 ab	15.55 a-d	11.81 g-j	548.44 b-f	35.33 b-e	16.77 a-d	15.38 abc	−11.84
12	70.88 hi	216.33 f-j	112.55 f-i	3.22 a-d	7.00 c	3.00 a-d	1.77 e-i	178.16 c-h	511.44 ab	15.11 a-d	11.61 hij	519.78 c-g	34.44 b-e	17.05 abc	14.90 abc	−13.32
13	71.55 ghi	228.44 a-g	109.66 ghi	3.11 a-d	11.00 a	3.88 a	1.55 f-i	172.38 d-h	428.78 b	12.66 e	10.49 ij	420.67 gh	33.22 c-f	15.31 d	15.33 abc	−21.65
14	70.11 ghi	218.55 f-i	105.33 hi	4.00 a	4.22 ghi	3.33 abc	3.88 a	159.22 gh	525.44 ab	17.11 a	11.80 g-j	604.67 abc	35.44 a-e	16.40 a-d	15.33 abc	−11.92
15	72.11 f-i	235.88 a-d	115.22 d-g	3.22 a-d	4.11 g-j	3.11 a-d	1.88 d-i	184.44 b-f	498.44 ab	13.77 cde	12.80 d-h	481.11 fgh	34.88 b-e	15.63 cd	16.44 ab	−4.44
16	72.33 fgh	224.00 c-g	127.55 bcd	2.88 cde	3.11 jk	2.55 b-f	2.11 d-i	186.88 b-f	600.44 a	14.44 b-e	15.61 a	540.00 b-f	37.33 abc	16.23 a-d	16.88 a	16.55
17	76.66 ab	241.00 a	128.00 bcd	2.66 c-f	6.66 dc	1.55 gh	1.33 hi	154.27 h	556.56 ab	16.00 abc	14.41 a-d	650.67 a	40.77 a	16.15 a-d	16.83 a	7.56
18	75.00 bcd	212.88 h-k	112.11 f-i	2.77 c-f	4.77 fgh	2.00 e-h	2.00 d-i	158.77 gh	519.56 ab	16.22 ab	13.50 c-f	570.22 a-f	35.22 b-e	15.80 bcd	15.47 abc	0.75
19	75.33 bcd	222.55 c-h	108.33 hi	2.88 cde	3.66 ij	2.77 b-e	1.44 ghi	206.66 ab	587.78 a	15.11 a-d	12.40 e-h	482.00 fgh	31.88 def	17.60 a	16.27 abc	−7.44
20	73.77 def	227.77 a-g	117.00 c-h	3.11 a-d	3.88 hij	3.11 a-d	1.88 d-i	197.77 abc	578.44 a	14.44 b-e	15.52 ab	500.44 d-h	34.66 b-e	16.70 a-d	16.14 abc	15.87
21	75.00 bcd	223.88 c-h	126.88 b-e	3 bcd	2.55 k	2.22 d-h	2.55 cde	182.11 b-g	583.33 a	15.77 abc	15.63 a	542.67 b-f	34.44 b-e	16.62 a-d	16.33 ab	16.69
22	74.77 cd	236.66 abc	146.11 a	3.00 bcd	6.44 dc	3.22 abc	2.00 d-i	189.55 b-f	575.11 a	15.22 a-d	14.56 abc	530.00 c-f	35.00 b-e	16.44 a-d	16.27 abc	8.69
23	76.00 abc	238.88 b-f	125.66 b-f	2.88 cde	3.66 ij	2.66 b-e	3.33 abc	190.22 b-e	595.56 a	16.22 ab	15.71 a	533.56 b-f	32.88 c-f	16.93 abc	16.05 abc	17.25
24	76.00 abc	235.55 bc	129.33 bc	3.00 bcd	3.77 hij	2.77 b-e	1.66 e-i	164.77 fgh	534.22 ab	15.77 abc	12.03 f-i	585.56 a-e	37.22 a-d	16.38 a-d	16.00 abc	−10.17
25	77.33 a	239.22 ab	135.00 ab	3.55 abc	5.88 de	3.33 abc	2.11 d-i	166.00 e-h	542.11 ab	14.66 b-e	13.30 c-g	568.67 a-f	38.88 ab	16.30 a-d	16.61	−0.75
DHS	1.80	14.61	14.00	1.00	1.10	0.92	0.90	24.95	135.36	2.27	1.63	99.66	5.43	1.48	2.29	

*-=Range of the means for each treatment, C=control; DMF=days to male flowering; PH=plant height; EH=ear height; HLM=Helminthosporium; FUS=Fusarium; R=rust; EC=ear coverage; W500G=500-grain weight; WGE=grain weight per ear; ROW=number of rows; YIELD=grain yield in tons per hectare; G×E=grains per ear; G×ROW=grains per row; PER=ear circumference; EL=ear length; %/CON=percentage relative to the average yield of the controls; TREAT=treatment; HSD=honest significant difference.

with 15.7, 15.6, and 15.6 t ha^{-1}, respectively, while hybrid 8 had the lowest yield with 10 t ha^{-1}. The hybrid with the most grains per ear was 17, with 650 grains, and the one with the fewest grains was 10, with 415 grains, a crucial characteristic influencing high and low yields in the genotypes. Regarding grains per row, hybrid 17 had 40 grains, while hybrid 8 had the fewest with 28 grains. For the perimeter of the ear (PER), hybrid 19 had the largest perimeter with 17.6 cm, and hybrid 13 had the smallest with 15.3 cm. Finally, concerning the length of the ear (EL), hybrids 16 and 17 had the greatest length, both with 16.8 cm, while the genotype with the shortest length was 8, with 14 cm.

Genotype-Environment Interaction Analysis

The analysis of variance (Table 4) showed a highly significant effect of the environment ($P \leq 0.01$), accounting for 30.2% of the total sum of squares (TSS). The genotype factor was also highly significant ($P \leq 0.01$), registering 53.3% of the TSS. The genotype-by-environment interaction was significant ($P \leq 0.01$), contributing 16.4% to the TSS. The AMMI model showed that the first two principal component axes were highly significant ($P \leq 0.01$), explaining 75.7% and 24.2% of the interaction sum of squares, respectively. The AMMI model retained 96% of the TSS (E+G+E*G) utilizing 51 degrees of freedom (2 for E, 24 for G, and 25 for the first principal component).

The results of the AMMI analysis facilitated the graphical representation (biplot) of genotypes and environments in the same space (Figure 4). On the abscissa axis (x), the grain yield of genotypes and environments is presented. The line perpendicular to this axis indicates the mean yield, which was 13 t ha^{-1}. Likewise, entries with lower yield are plotted to the left of the X-axis, while genotypes and environments with higher yield are located to the right.

The Y-axis, on the other hand, measures the stability of genotypes and environments: those with values close to zero are stable, while those with high values of the first principal component are unstable. According to this information, genotypes 23, 21, 16, and 20 achieved the highest yields, followed by genotypes 22, 6, 17, and 5. In contrast, genotypes 8 and 13 showed the lowest yields, these data are consistent with the averages mentioned in Table 3. The locality of Comonfort achieved the highest yield, followed by Soria; the locality of Juventino Rosas recorded yields below the average. On the other hand, the most

Table 4. Analysis of Variance of the AMMI Model for 25 Maize Hybrids Evaluated in 3 Environments.

S.V.	D.F	S.S	% TSS
Environment (E)	2	309 **	30.21
Genotype (G)	24	546 **	53.32
G*E	48	169 **	16.47
PC1	25	128 **	75.75
PC2	23	41 **	24.25

S.V.=Source of variation; DF=Degrees of Freedom; SS=Sum of Squares; %TSS=percentage of total sum of squares; G*E=genotype by environment interaction; PC=Principal Component.

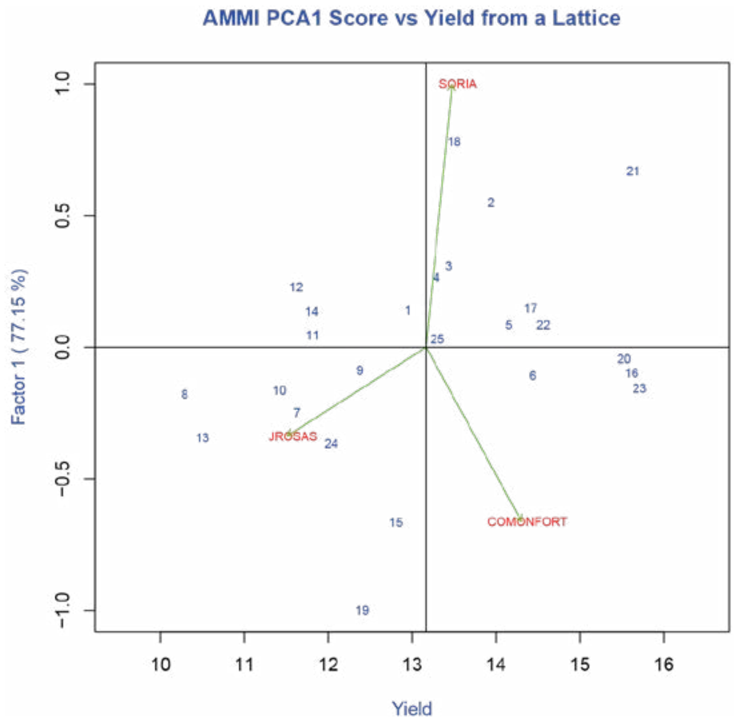

Figure 4. Biplot of grain yield for 25 maize hybrids evaluated in the experiment.

stable genotypes, with low or near-zero PC1 values, were genotypes 20, 16, 23, 17, 22, 6, 5, 25, 4, 1, 9, 11, 14, 7, and 8. However, genotypes 20, 16, and 23 stood out the most in terms of yield, indicating that these materials performed well across all environments. Genotypes 21, 18, and 19, along with the Soria environment, contributed the most to the first axis of interaction, making them the most unstable. Regarding the environments, Yan *et al.* (2000) notes that those with an angle less than 90° between them tend to classify genotypes in a similar manner, while those with an angle close to 180° tend to order genotypes inversely, making material selection more challenging due to their contrasting nature, as observed in the environments of Soria and Comonfort. Given the length of the vectors, the environments that best discriminated the genotypes in the evaluation were Soria and Comonfort, according to was explained by Kempton (1984).

CONCLUSIONS

There is high genetic divergence in the expression of yield and phenological parameters. The outstanding hybrids in this experiment were 23, 21, and 16, as they revealed the best yields across all locations.

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Colegio de Postgraduados

The Seeds of the Mexican Countryside

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ABSTRACT

Objective: The objective of this work is to make an analysis of the types of seeds use by Mexican farmers and their relationship with Local Seed Systems (SLS) and Formal Seed Systems (SFS).

Design/methodology/approach: The work consisted of a bibliographic review about the types of seeds available for the Mexican countryside and their seed systems. In addition, to identify some priorities and strategies for its strengthening.

Results: In Mexico, derived from its climatic, edaphic, geophysical heterogeneity, cultural social and economic diversity. It has caused both landraces and improved seed to coexist in the SLS and SFS. However, there is a predominance in the use of landraces in SLS of 70 to 80%. Therefore, it is considered a priority to conserve traditional selection, management, conservation and use practices that have caused the wide inter- and intraspecific diversity of landraces. However, more research needs to be carried out and its legal framework strengthened. In the case of improved seeds located mainly in SFS, it is considered to promote their use in vegetables and the ornamental sector.

Limitations on study/implications: The work developed could be complemented with the participation and interviews of decision makers in seed policies in Mexico and the review of processed in other countries.

Findings/conclusions: In Mexico the use of native and improved seeds coexists. To satisfy the demand for food, all types of seeds are important and both the SLS and the SFS but differentiated public policies are required for their attention and the definition of a specific legal framework for landraces.

Keywords: improved seed, landraces, selection, management.

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INTRODUCTION

Seeds are one of the main inputs for agricultural production. Having quality seeds, culturally relevant and adapted to different ecological niches is the foundation for food security and sovereignty (Kuhlmann and Dey, 2021). In Mexico, according to the Federal Law on Seed Production, Certification, and Commerce, seeds are defined as: "those obtained from the fruit after the fertilization of the flower, the fruits or parts thereof, as well as parts of plants or whole plants used for the reproduction and propagation of different plant species" (DOF, 2018). In Mexico, farmers mainly use native and improved seeds. This last one is generally certified by the SNICS (National Service of Seed Inspection and Certification), which is responsible for coordinating activities related to the production, certification, and trade of seeds (SAGARPA, 2005). According to the seed law, seeds are categorized as: certified, approved, and declared (DOF, 2018). These are multiplied from original (or genetic), basic registered, and certified seed categories. However, landraces can also achieve these categories if the varieties are registered in the National Catalogue of Plant Varieties (CNVV) (DOF, 2018; Domínguez-García *et al.*, 2019; Escárraga-Torres *et al.*, 2021), as explained below.

Types of Seeds Used by Mexican Farmers

In Mexico, different types of seeds are used by farmers (Domínguez-García *et al.*, 2019; Escárraga-Torres *et al.*, 2021). The type of seed they use depends on soil conditions, climate, geophysical factors, water availability, land tenure, surface area size, production potential, marketing channels, type of agroecosystem, among other factors (Almekinders and Louwaars, 1999; Thijssen *et al.*, 2008). The main types are:

Landraces

Landraces is defined as seed that is selected, managed, and preserved by the farmers themselves. It is adapted to the specific conditions of each ecological niche. Additionally, over the years, farmers select characteristics according to their needs and uses (Hernández-Sandoval *et al.*, 2023). These seeds can be grouped into races or varietal groups, which have been shown to share their evolutionary history but are not completely homogeneous because the process of obtaining them is dynamic and continuous (CONABIO, 2024a). However, due to management practices, curiosity, or interest from the farmers, as well as natural biological aspects, landraces can be combined with improved seeds, closely related wild relatives, or materials from neighboring farmers. This generates a wide intra-specific diversity (Bellon, 1996; Casañas *et al.*, 2017). Most studies have documented this interaction between different types of seeds and their management, selection, and conservation in maize (Bellón, 1996). However, this is not well understood for other crops important to Mexican food and agriculture, such as beans, squash, and chili peppers, among others. Therefore, landraces are fundamental for generating more inter- and intra-specific diversity, serving as the basis for food production for self-consumption. Moreover, they are adapted to conditions of biotic and abiotic stress that improved seeds may not be able to tolerate (Almekinders and Louwaars, 1999; Hernández-Rodríguez *et al.*, 2020). Farmers produce their own landraces for each agricultural cycle (CONABIO, 2024a; Hernández-Sandoval *et al.*, 2023). According to INEGI (2019), the use of landraces in Mexico represents 61.77%.

It is considered that there is no legal framework for the conservation, strengthening, legal protection of seeds, and the rights of farmers who have generated this diversity for thousands of years (Domínguez-Martínez *et al.*, 2019; Escárraga-Torres *et al.*, 2021). Exceptions are made for native maize, which, according to Escárraga-Torres *et al.* (2021), can be seen as a milestone with the Federal Law for the Promotion and Protection of Native Maize. This law aims to consider the activities of production, commercialization, and consumption of native maize as a cultural manifestation and an obligation of the State to guarantee the right to nutritious, sufficient, and quality food, and to define the mechanisms for the protection and promotion of maize (DOF, 2020a). Some states have similar laws, such as Michoacán, with the Law for the Promotion and Protection of Creole Maize as the Food Heritage of the State of Michoacán de Ocampo (Official State Gazette, 2011), and Tlaxcala with the Law for the Promotion and Protection of Maize as Original Heritage, in Constant Diversification, and Food Heritage for the State of Tlaxcala (Official State Gazette of Tlaxcala, 2011). Similarly, due to its importance in food and other uses, it is necessary to document the wide intra-specific diversity of the seeds of crops introduced in Mexico, a situation similar to that occurring worldwide (Pilling *et al.*, 2020). For example, Hernández-Sandoval *et al.* (2023) identified five types of broad beans with different common names and morphologies in indigenous communities of Amealco, Querétaro. These are diversifying through the management of the farmers. Therefore, it is very likely that seed populations of introduced species may disappear due to environmental, social, or economic factors without being documented. Additionally, Mexico does not have official statistics on the production of intra-specific diversity of any crop (SIAP, 2023). For example, there are no statistics on maize production by race.

In Mexico, it is possible to produce certified seed (a category explained in the following section) from landraces, as long as the variety as registered in the National Catalogue of Plant Varieties (CNVV). Among the necessary requirements is morphological characterization based on the technical guidelines for varietal description outlined in the Federal Law of Plant Varieties, later referred to as the Law of Plant Varieties (Domínguez-Martínez *et al.*, 2019; SNICS, 2024d). Therefore, in Mexico, according to the legal framework, a Mexican farmer can plant landraces with the certified, approved, and declared categories considered in the Seed Law (DOF, 2018), which has not yet been fully implemented in the country. This is because it is generally small farmers who possess such seeds and lack the technical capacities and infrastructure to carry out the registration. To encourage the registration of native varieties, public agricultural research institutions must advise and support the producers to achieve this registration.

It should be noted that common-use varieties have been registered in the CNVV with the aim of documenting the inter- and intra-specific diversity of native crops (González-Santos *et al.*, 2015). Additionally, having records of the origin of the variety helps prevent potential cases of piracy (Domínguez-Martínez *et al.*, 2019). Notable registrations include 61 common-use varieties of nopal, 30 of marigold (*Tagetes* L.), 30 of xoconostle (*Opuntia* spp.), and 10 of pitaya (*Stenocereus* spp.), among others (SNICS, 2024b) (Table 1). However, given the diversity that exists in Mexico, this is considered not to be representative.

Improved Seed

Improved seed refers to seed obtained through any method of genetic improvement. These seeds are defined as new, homogeneous, stable, and with distinctive characteristics. They are generated for specific conditions of altitude, soil, water availability, nutrients, and uses (Bellon, 1996; Zeven, 1999; Casañas *et al.*, 2017; Domínguez-Martínez *et al.*, 2019). Generally, this seed is used in irrigated areas and areas with good rainfall, with farmers who have financial resources, often associated with large land holdings and agro-industrial crops such as sorghum, barley, and wheat. The main regions using this seed

Table 1. Common-Use Varieties Registered in the National Catalogue of Plant Varieties by SNICS. Prepared with data from SNICS.

Crop	Number of varieties
Agave (*Agave* spp.)	12
Cempoalxóchitl (*Tagetes* L.)	30
Chayote [*Sechium edule* (Jacq.) Sw.]	10
Dahlia (*Dahlia* Cav.)	6
Guava (*Psidium guajava* L.)	5
Nopal (*Opuntia* spp.)	61
Poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch)	3
Pitahaya [*Hylocereus undatus* (Haw.) Britton & Rose]	2
Pitaya (*Stenocereus* spp.)	10
Hawthorn (*Crataegus* L.)	5
Tigridia [*Tigridia pavonia* (L. f.) Redouté]	9
Echeveria (*E. gibbiflora* DC. y *E. pallida* E. Walther)	4
Xoconostle (*Opuntia* spp.)	30

in Mexico are Sinaloa, Jalisco, Tamaulipas, certain regions of the Highlands, Bajío, and the Altiplano (DOF, 2020b). According to INEGI (2019), 24.88% of the seed planted in Mexico is certified improved seed, while the rest is landraces.

SNICS is responsible for ensuring compliance with the requirements established in the Rules for Seed Qualification, specific to each crop, from field establishment, storage, and commercialization (Córdova-Téllez *et al.*, 2017; Domínguez-Martínez *et al.*, 2019). Quality parameters are related to physiological, phytosanitary, genetic, and physical quality. Physiological quality refers to the seed's ability to produce viable propagation material; phytosanitary quality refers to the assessment and determination of the presence or absence of pathogenic organisms in the seed lot; genetic quality refers to the genetic identity of the seed; and finally, physical quality refers to the measure of the seed's physical purity (DOF, 2018).

SNICS has 33 rules for seed qualification (Table 2) (SNICS, 2024c). Therefore, the necessary instruments are available only to certify seeds of these crops. If there is interest in another crop, it is first necessary to define the technical rule for seed qualification according to the "Official Mexican Standard NOM-001-SAG/FITO, 2015, which establishes the criteria, procedures, and specifications for the preparation of guides for varietal description and rules for determining the quality of seeds for planting" (DOF, 2015).

The qualification rules mentioned in Table 2 highlight that there is no rule for the qualification of seeds for certain crops that Mexico exports, such as: tomato (*Solanum lycopersicum*), berries (various species), broccoli (*Brassica oleracea*), lemon (*Citrus*×*limon*), lettuce (*Lactuca sativa*), cucumber (*Cucumis sativus*), and asparagus (*Asparagus officinalis*) (SIAP, 2023). This aligns with the National Seed Program published in the DOF (2020b), which states that most vegetable seeds are imported. It is considered necessary

Table 2. Rules for Seed Qualification, in accordance with international standards issued by SNICS.

Cereals	Forage	Fruit	Vegetables	Industrial	Ornamental
Oats (*Avena sativa* L.)	Alfalfa	Avocado	Garlic	Sesame	Chrysanthemum
rice (*Oryza sativa* L.)	Pastures (11 species)	Coffee	Pumpkin	Cotton	Rose
Cereals (oats, barley, rye, wheat and triticale	Sorghum	Coconut tre	Onio	Peanut	
Chickpea		Nopal	Pepper	Canola	
Corn		Papaya	Potato	safflower	
Millet			Husk tomato	Bean	
Wheat				Sunflower	
				Faba bean	
				Castorbean	
				Soja	

to conduct a specific study of vegetable seeds to understand the mechanisms that farmers use for planting. The ornamental sector is also relevant for Mexico, as approximately 14,936 hectares are dedicated to ornamental horticulture, with a production value of $5,192,931,852.89. The most produced species are rose (*Rosa* spp.), sunflower (*Helianthus annuus*), gerbera (*Gerbera jamesonii*), and lily (*Lilium candidum*). In the case of roses, in 2022 they were cultivated in 10 states, with a production of 10 million 37 thousand dozen (SIAP, 2023). However, technical rules for the qualification of seeds are only available for roses and chrysanthemums, and ornamental seeds are still not certified in Mexico (SNICS, 2024). In the opinion of the authors of this article, it is likely that, by integrating the seed certification process, cases of illicit use of varieties with national and international breeder's certificates will decrease.

Based on historical records from 1988 to 2023, the production of certified seed has not exceeded 350,000 tons. The years with the highest production of certified seed were 1989 with 340,000 tons, 2008 with 300,000 tons, and 2014 with 325,000 tons. Some of the factors that have influenced production include the disappearance of Seed National Producer (Productora Nacional de Semillas - PRONASE), the involvement of the private sector, and government programs that give preference to certified seeds, among others (Domínguez-Martínez *et al.*, 2019; SNICS, 2019). Additionally, not all crops that have a technical rule for seed qualification are certified. For example, chrysanthemums (*Chrysanthemum coronarium*), roses (*Rosa* spp.), castor beans (*Ricinus communis*), and squash (*Cucurbita* spp.) can be produced in the declared category. On average, seeds from 22 crops are certified (Domínguez-Martínez *et al.*, 2019; Córdova-Téllez *et al.*, 2018). For instance, during the spring-summer 2023 agricultural cycle, the production of certified seed was 47,068.46 tons from 142 varieties including rice, oats, peanuts, coffee, barley, beans, corn, Brachiaria grass, sorghum, soybeans, and triticale (SNICS, 2024b).

The seed law also includes the category of habilitated seed, which farmers can use for planting. It is defined in the law as "seed whose propagation or production process has not been verified, or if verified, does not fully meet any of the genetic, physical, physiological, or phytosanitary quality characteristics, with tolerance parameters defined in the specific Rules for Seed Qualification of each crop" (DOF, 2018). Another type of seed available to Mexican farmers for planting improved seeds is the declared category. In this category, the characteristics are not evaluated by SNICS; they are provided directly by the producer or seller on the label with the requirements established in Article 33 of the Seed Law. This means that the label must include information such as the variety name, germination percentage, and purity percentage (DOF, 2018; Domínguez-Martínez *et al.*, 2019).

Genetically Modified Seed

According to INEGI (2019), only 0.18% of the seeds planted in Mexico are genetically modified, with cotton and soybeans being the primary species. The use of this type of seed is regulated by the Law on Biosafety of Genetically Modified Organisms (DOF, 2022) and undergoes a series of processes and tests before it can be used by farmers. No new permits have been issued since 2019; prior to that year, permits were authorized for cotton, wheat, alfalfa, Mexican lime, Valencia sweet orange, beans, soybeans, and corn (CIBIOGEM, 2024). From 2018 to 2024, there has been ongoing discussion about the possibility of banning the use of genetically modified organisms in Mexico, specifically for corn.

Distribution of Seeds in Different Seed Systems

Authors such as Domínguez-Martínez *et al.* (2019), Torres *et al.* (2014), and DOF (2022b), mention the types of seeds used in two main systems: the Local Seed System (SLS) and the Formal Seed System (FSS), which are explained below.

Local Seed System

In the Local Seed System (LSS), landraces are generally used in all the hybridizations (Castillo and Goodman, 1995; Casañas *et al.*, 2017). In this system, within the same cycle and in the same area, seeds are obtained for the next planting cycle, while the grain is used for self-consumption or commercialization. As a result, the farmer is self-sufficient in seed production (Almekinders and Louwaars, 1999). Additionally, seeds are selected according to needs and uses. Exchange and access occur through customs and traditions. In the SLS, seeds can be inherited from parents, borrowed, purchased, or given by neighbors or family members. In fact, it is possible for farmers to obtain seeds at no cost (Bellon, 1996; Thijssen *et al.*, 2008). It is estimated that this system in Mexico represents between 70 to 80% (INEGI, 2019). However, it faces several issues, such as some native varieties having characteristics that represent disadvantages for farmers. For example, low yield, susceptibility to lodging, long cycles, among others. These issues should be addressed with participatory plant breeding programs in the short, medium, and long term, according to the specific needs of the regions. Sustainable alternatives for the storage of seeds and grains are also needed to prevent loss or the use of toxic chemicals. Additionally, there are social and economic factors leading to the decline of this system, such as the aging of farmers without generational replacement, lack of interest from young people in agriculture, migration, and changes in family activity organization (Louwaars & Simon de Boef, 2013). For example, in the Otomi communities of Amealco, Querétaro, it is noted that what was once a family-wide activity is now maintained only by elderly individuals (Hernández-Sandoval *et al*., 2023).

It should be considered that the Local Seed System (LSS) consists of various types of agroecosystems present in Mexico. CONABIO (2024b) identifies at least 30 different types, such as milpas, terraces, chinampas, family gardens, and backyards. However, a large part of the country remains undocumented. In these, different seed sources, management methods, conservation types, and even species with varying degrees of domestication are used. It is agreed with Escárraga-Torres *et al.* (2021) that the LSS and its seeds have been little studied, with significant gaps in the legal framework. This coincides with Bautista-Ramírez *et al.* (2022), who mention that research on seeds is mainly focused on physiological, sanitary, and physical quality, with few studies related to conservation, management, selection, and diversity.

Formal Seed System

The FSS in Mexico is primarily supported by the Seed Law and the Plant Variety Law. In this system, improved seeds are used, which can either undergo the certification process (certified seed) or not (declared seed) (Domínguez-Martínez *et al*., 2019). It is likely that 0.8% of transgenic seed, as indicated by INEGI (2019), is used in this system. The FSS is associated with specific regions of the country and is used for monoculture production. This system depends on the LSS to obtain the seed from which genetic improvement programs are carried out, as described by Almekinders and Louwaars (1999). The disadvantage is that farmers become dependent on this input, having to purchase seed for each planting cycle (Thijssen *et al*., 2008). Additionally, the FSS has drawbacks, such as weak or nonexistent cultural ties with consumers. Its cultivation requires large energy investments, as well as specific distribution methods (Casañas *et al*., 2017; Zeven, 1999). The FSS faces difficulties in meeting the needs of small farmers with limited resources and in marginal conditions because these farmers usually require small quantities, which represents a challenge in seed distribution (Zeven, 1999; Bellon, 1996; Thijssen *et al*., 2008). Additionally, the demand for seeds varies significantly between agricultural cycles, depending on the production of the previous season and the economic resources available to farmers (Hernández-Rodríguez *et al*., 2020).

Coexistence of Seed Systems in Mexico

In Mexico, both the Local Seed System (LSS) and the Formal Seed System (FSS) coexist, with the former being predominant. This dominance may be attributed to the country's climatic, edaphic, and geophysical heterogeneity (Llorente and Ocegueda, 2008). Additionally, the cultural diversity with the presence of 62 indigenous groups contributes to a wide range of ecological niches where various local varieties have evolved and adapted. This has led to Mexico being the center of domestication for 251 species (Clement *et al*., 2021), as well as the different methods of management, selection, and conservation primarily implemented in the LSS. In contrast, the FSS is limited to specific areas of the country as previously mentioned. However, most federal and state government efforts focus on providing improved seeds to farmers.

The latter may not adapt to the conditions faced by farmers in the LSS and could lead to the loss of landraces due to substitution. The National Seed Program (DOF, 2020b) proposed increasing the use of improved seeds in the country, but it is considered that this should not be generalized. Priorities should be defined; for example, it may be a priority for export vegetables and the ornamental sector. However, in the SLS, it is necessary to promote the use of landraces and preserve traditional practices of selection, management, conservation, and exchange that have led to the noted intra-specific diversity. Both systems are considered important for ensuring food production, but they have specific seed requirements and face different issues that need to be addressed separately.

CONCLUSIONS

In Mexico, farmers primarily plant landraces and, to a lesser extent, improved seeds, which can be certified, authorized, or declared according to the seed law. Landraces are generally used in the Local Seed System, while improved seeds are used in the Formal Seed System. Therefore, there is a coexistence of systems. Each presents different needs in terms of seed type and faces specific ecological, social, economic issues, and gaps in the legal framework. Thus, differentiated public policies are required for both types of seed systems to ensure food security and sovereignty for Mexicans.

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Colegio de Postgraduados

Agroecological Alternatives for Pest and Disease Management in Mexican Lime [*Citrus aurantifolia* (Christm.) Swingle] Cultivation

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ABSTRACT

Objective: To evaluate the effect of commercial bioinsecticides and mineral broths as agroecological alternatives for the integrated management of recurrent pests in Mexican lime (*Citrus aurantifolia*); as well as to establish possible synergies with the commercial bioinsecticide.

Design/methodology/approach: This experiment was conducted in two phases: 1) visual monitoring and pre-identification of pests and fungal diseases in the Mexican lime crop, and 2) application and evaluation of the effect of application of commercial bioinsecticide (Biocanela) and mineral broths (bordeaux broth and sulfocalcium broth) alone and mixtures.

Results: The pests with highest incidence were thrips (*Pezothrips kellynus*), Asian citrus psyllid (*Diaphorina citri*), whitefly (*Bemisia tabaci*), fruit fly (*Drosophila melanogaster*), and red spider mite (*Tetranychus urticae*), while the main fungal diseases were sooty mold (*Capnodium citri*), red algae (*Cephaleuros virescens*), citrus greasy spot (*Mycosphaerella citri*), and anthracnose (*Colletotrichum acutatum*). The mixture of mineral broths with the bioinsecticide Biocanela showed the highest fungicide and insecticide activity, and repellency on various pests and diseases of the Mexican lemon crop.

Limitations on study/implications: Generate scientific knowledge regarding to the best agroecological alternatives for the integrated management of citrus pests and diseases that are economically and environmentally profitable.

Findings/conclusions: Application of the mixture of sulfocalcium broth and Biocanela showed repellency effectiveness against pests such as thrips (*Pezothrips kellyanus*), Asian citrus psyllid (*Diaphorina citri*), whitefly (*Bemisia tabaci*), and fruit fly (*Drosophila melanogaster*). Likewise, it was effective against sooty mold (*Capnodium citri*), red algae (*Cephaleuros virescens*), and citrus greasy spot (*Mycosphaerella citri*), while the effect was lighter against anthracnose (*Colletotrichum acutatum*). Additionally, it induces new, healthy shoots in Mexican lemon trees.

Keywords: mineral broths, commercial bio-insecticides, citrus, phytopathology

Image by Alejandro Sánchez

INTRODUCTION

Lime cultivation in Mexico is carried out in 27 states, where three varieties are grown: Mexican lime [*Citrus aurantifolia* (Christm.) Swingle], Persian lime (*C. latifolia* L.), and Italian lemon (*C. lemon* L.). Among these, Mexican lime has the largest area of commercial orchards (Martínez *et al.*, 2023), with an annual production of approximately 3.1 million tons valued at 28,141 million pesos (SIAP, 2022). The production of lime in all its varieties developed during the 20th century, with the first plantations starting in the state of Michoacán. This was done to meet the demand for fresh lime and lime oil in the U.S. and French markets, as well as the growing local consumption (Galván-Vela and Santos-González, 2019). Currently, Mexican lime cultivation covers 80% of the cultivated area in the state of Colima, with Tecomán and Armería being the municipalities with the largest cultivated areas (Orozco-Santos *et al.*, 2014). However, the tropical climate in which this crop is predominantly grown fosters the presence of pests and diseases, making agroecological alternatives necessary for the management of these pathologies.

The use of synthetic pesticides for pest control in citrus has been the primary option for managing phytopathogens, despite their residuality and high costs, as well as the potential for pests to develop tolerance (González-Castro *et al.*, 2019). Synthetic pesticides are made from chemical products such as specific polymers for different pests; these include those used for weed control (herbicides), fungi (fungicides), mites (acaricides), bacteria (bactericides), insects (insecticides), nematodes (nematicides), etc., which form the basis of their classification (Anakwue, 2019; Rakhimol *et al.*, 2020). Although synthetic pesticides have positive effects on crop yield and productivity, they also have some negative impacts on soil biodiversity, wildlife, and aquatic life (Faroq *et al.*, 2019). Similarly, they affect soil microorganisms by limiting their biological services in the production of certain plant growth-promoting traits, such as siderophores, nitrogen, and indole-3-acetic acid, among others (Kumar and Kumar, 2019).

In the past 20 years, the development of production systems, technologies, and integrated pest management plans that reduce or eliminate these problems has shown rapid growth worldwide (Seufert *et al.*, 2017). In this regard, technological alternatives for integrated pest management include the use of bioinsecticides and mineral broths (Tijjani *et al.*, 2016). Bioinsecticides are natural substances composed of living organisms that control pests through mechanisms with minimal negative impact on the environment (Mazid *et al.*, 2011); meanwhile, mineral broths are liquid organic fertilizers that act as fungicides, insecticides, and acaricides while also providing nutritional benefits to crops (Leal and Reyes, 2015).

In Mexico, statistics on the use of agroecological alternatives such as bioinsecticides and mineral broths show widespread use in crops like avocado, corn, and chickpeas (Bouriga-Valdivia *et al.*, 2016). Specifically, in the state of Colima, the use of these bioinsecticides is still limited, with their primary application being in banana cultivation (Arévalos, 2017). The objective of this study was to evaluate commercial bioinsecticides and mineral broths as agroecological alternatives for the integrated management of recurrent pests in Mexican lime cultivation, as well as to establish possible synergies with the commercial bioinsecticide.

MATERIALS AND METHODS

Experimental Area and Biological Material

The experiment was conducted in the community of Cofradía de Juárez in the municipality of Armería, Colima, located at coordinates 18° 59' 44" North latitude, 103° 55' 45" West longitude, at an altitude of 46 meters (INEGI, 2019; Google Earth, 2019). The property borders a low deciduous forest area to the south (INEGI, 2019), and to the north, it is adjacent to the main unpaved road without a dust protective fence. The land area is one hectare with a planting density of 400 Mexican lime trees, averaging 5 to 6 years old, and a previously established planting frame of 6×7 meters. The study included two stages: the first involved monitoring and preliminary identification of pests and fungal diseases present in the crop, and the second involved the application of the specified treatments to each row of the crop.

Monitoring and Identification of Pests and Fungal Diseases in Mexican Lime Cultivation

Monitoring was carried out during the first 15 days of the experiment in November and December. The experimental area was divided into five quadrants (four at each corner and one in the center forming a "five of coins" pattern), and a yellow trap measuring 12.5×21.5 cm, with thick gauge and glue on both sides from the *Ferommis Group* (Culiacán, Sinaloa), was placed at the center of each quadrant. Every 15 days during the monitoring period, different sides of the traps were examined to accurately determine the pests and their densities.

Pest identification was done visually, while density was determined using the guidelines provided by the trap supplier during the counting of insects in the grid sections of each trap. Fungal disease identification was also conducted visually, through monitoring 15 randomly selected trees in one of the rows corresponding to each treatment.

Treatment of the Crop and Pest Sampling

The experimental stage included cycles of spraying and pest monitoring. Eight treatments with three replications were used for the control of fungal infections and pests. The treatments were: absolute control, control 1 (commercial chemical insecticide: Bannen 1.8%), the bioinsecticide (Biocanela), sulfocalcium broth+bioinsecticide (Biocanela), sulfocalcium broth, Bordeaux broth+bioinsecticide (Biocanela), Bordeaux broth, control 2 (commercial chemical fungicide: Funlate 50%) (Table 1). Before application, all products were mixed with an adjuvant (Bionex®). The active ingredients and the doses corresponding to each treatment are shown in Table 1. The trees selected for pest monitoring (15 per treatment) were evaluated directly (visually) to determine the incidence of fungal pathologies in four zones corresponding to the cardinal points, monitoring the trunk, primary branches, secondary branches, and fruiting branches in each zone. Applications were made using a 20 L motor pump (Mitsubishi F-767) (Duran-Trujillo *et al.*, 2017). Spraying was conducted from February to April, with one application every 15 days throughout the experimental period (6 months). Each tree was sprayed according to the cardinal points with the eight treatments; subsequently, the trees were labeled with

Table 1. Treatments and doses evaluated for pest control in Mexican Lime during the experiment.

Treatments	Name	Active ingredient	Dose (L ha^{-1})
T1 (absolute control)	Water	H_2O	200
T2 (control sample 1)	Funlate 50%	Benomyl	300
T3	Biocanela	Cinnamon oil	1.5
T4	Sulphocalcium broth + Biocanela	Sulfur + calcium oxide + cinnamon oil	40 + 1.5
T5	Sulphocalcium broth	Sulfur + calcium oxide	40
T6	Bordeaux broth + Biocanela	Copper sulfate + hydrated lime + cinnamon oil	40 + 1.5
T7	Bordeaux broth	Copper sulfate + hydrated lime	40
T8 (control sample 2)	Bannen 1.8%	Abamectin	1.5

the treatment name, time, and date to track the applications. Additionally, one yellow trap from *Ferommis Group* (Culiacán, Sinaloa) was placed per treatment and replication. The 24 traps were placed randomly within each replication. To track population fluctuations of the species and measure the effect of the treatments, monitoring was conducted 5, 7, 9, 11, and 13 days after each application. The adjuvant Bionex® was added to all treatments at a concentration of 1 L ha^{-1}.

Experimental Design

A completely randomized mixed effects generalized linear model with three replications was used. The effects included in the model were: number of insects per trap before application, number of insects per trap after application, and number of insects killed on the branches. The direct observation method was used to determine the fungicidal effectiveness of the mineral broths.

Data Analysis

The reductions in pest populations due to the treatments were calculated by comparing them with the number of insects in the control plot (Duran-Trujillo *et al.*, 2017). The percentage of reduction (R%) in infestation was estimated using the Henderson and Tilton (1955) equation with the SAS 9.3 statistical package, through the following formula:

$$Infestation\ percentage = \sum \left(\frac{n.v}{N.V.} \right) \times 100$$

Where n=number of monitoring units in each category, N=total number of monitoring units, v=value of each category, and V=value of the highest category.

The results were transformed using the arcsine function and subsequently analyzed with SAS 9.3 software. For the comparison of treatments, analysis of variance (ANOVA) was used, and mean comparisons were conducted with the Tukey test. For correlation, the Pearson test was employed. In all cases, $\alpha=0.05\%$ (Duran-Trujillo *et al.*, 2017).

The behavior of the accumulated incidence (AI) of each fungal pathology due to the treatments was determined using the formula employed by Fajardo-Gutiérrez (2017):

$$Accumulate\ Incidence\ of\ Fungal\ Pathology\ (\%) = \frac{\#\ infected\ plants}{total\ number\ of\ monitored\ plants} \times 100$$

To determine the AI in each application cycle, the average number of diseased trees identified during the monitoring was used, while the AI for the entire study was based on the infected plants reported in the final monitoring conducted during the study.

RESULTS AND DISCUSSION

The pests with the highest initial presence recorded on the sampled trees were thrips (*P. kellyanus*): 260, Asian citrus psyllid (*D. citri*): 130, whitefly (*B. tabaci*): 277, fruit fly (*D. melanogaster*): 185, and red spider mite (*T. urticae*): 76; additionally, fungal diseases observed in trees included anthracnose (*C. acutatum*): 79, red algae (*C. virescens*): 118, sooty mold (*C. citri*): 114, and citrus greasy spot (*M. citri*): 81; all of which have a significant economic impact on the development of citrus crops. The effectiveness of the tested treatments for implementing integrated pest and disease management were different (Table 2).

The significant difference in the number of applications indicates that the insecticidal effect of the treatments used in this study begins with the second application (Table 3), while the fungicidal effect is evident only by the third application (Table 6), with its effectiveness

Table 2. Significance level for the effects included in the models to analyze the insect-trap ratio (ITR) and dead insects on trees (DIT).

Variable	$Application_z$	Day_y	$Treatment_x$	$Rep\ (Trat)_w$
ITR	<0.0001	<0.0001	<0.0001	<0.0001
DIT	<0.0001	<0.0001	<0.0001	0.9999

Rep (Trat): repetition nested within treatment.

Table 3. Tukey's mean comparison test for the overall effect by application of mineral broths and commercial products on insect-trap relationship (ITR) and insect mortality (DIT) in Mexican lime trees after five application cycles.

Application*	N	ITR[¥]	DIT[¥]
1	120	95.98 ± 38.40^{a}	10.82 ± 14.84^{a}
2	120	76.30 ± 37.78^{b}	9.79 ± 13.51^{ab}
3	120	71.90 ± 39.09^{b}	10.14 ± 12.62^{ab}
4	120	54.10 ± 32.01^{c}	8.50 ± 10.63^{bc}
5	120	49.08 ± 27.65^{c}	6.38 ± 8.61^{c}

ITR: insect-trap ratio; DIT: Insects Mortality in trees, *Interval between applications: 15 days, [¥]Values with the same letter within each column are not significantly different ($p<0.05$).

increasing as the number of applications progresses (Tables 3 and 6). Additionally, it was demonstrated that the repellent, insecticidal, and fungicidal behavior exhibited by all treatments does not change with subsequent applications, although the magnitude of the effect does.

It was determined that the number of days elapsed since the application of each treatment influenced the effectiveness of mineral broths and commercial products in terms of insect-trap relationship and insect mortality in trees, with partial pest control maintained up to nine days post-application (Table 4). A significant difference in repellent effectiveness was observed during the 13 days following the first application. However, the difference in its insecticidal behavior from day five to day 13 post-application was significantly low.

The combinations of Bordeaux mixture (BM) with Biocanela (CBR+BC) and sulfur-calcium broth with Biocanela (SCB+BC) generally exhibited the highest effectiveness as repellents, with insect-trap ratios of 46.85 and 53.80, respectively, and a reduction in overall pest incidence ranging between 71% and 79% (Table 5). Both broths exhibited significant repellent action against whitefly (WF), showing reductions ranging from 54% to 74%, along with treatment 5 of sulfur-calcium broth (Table 5 and 6). Similarly, in terms of pest type, the SC+BC treatment was found to be the most effective repellent for thrips

Table 4. Tukey's Mean Comparison Test of the General Effect of Days Post-Application (DAA) of Mineral Broths and Commercial Products on the Insect-Trap Ratio (ITR) and Insects Mortality (DIT) in Mexican lime Trees for Each Application Cycle.

DAA	N	ITR[¥]	DIT[¥]
5	120	54.45 ± 29.93^{a}	25.63 ± 17.57^{a}
7	120	63.09 ± 33.83^{b}	9.93 ± 6.69^{b}
9	120	69.52 ± 37.65^{c}	5.52 ± 3.83^{c}
11	120	76.65 ± 41.91^{d}	3.03 ± 2.58^{c}
13	120	83.67 ± 43.59^{e}	1.53 ± 1.67^{c}

DAA: days Post-Application; ITR: insect-trap ratio; DIT: Insects Mortality in trees, [¥]Values with the same letter within each column are not significantly different ($p<0.05$).

Table 5. Tukey's mean comparison test of treatments based on mineral broths and commercial products on insect-trap ratios and insect mortality in Mexican lime trees after five application cycles.

Treatments		N	IMI	ITR[¥]	DIT[¥]
T1	Water (absolute control)	75	211.80	135.88 ± 31.45^{a}	0.00 ± 0.00^{e}
T2	Funlate 50% (benomyl) control sample 1	75	189.30	80.29 ± 22.14^{b}	0.02 ± 0.16^{e}
T3	Bio-canela	75	197.73	55.96 ± 22.80^{d}	9.01 ± 8.83^{cd}
T4	Sulphocalcium broth + Bio-canela	75	192.40	53.80 ± 30.02^{de}	13.05 ± 13.29^{b}
T5	Sulphocalcium broth	75	190.67	66.21 ± 36.99^{c}	12.98 ± 12.87^{b}
T6	Bordeaux broth + Bio-canela	75	197.07	46.85 ± 18.43^{e}	11.77 ± 12.18^{cb}
T7	Bordeaux broth	75	205.00	60.02 ± 24.30^{cd}	8.52 ± 9.14^{d}
T8	Bannen 1.8% (abamectin) control sample 2	75	188.93	56.77 ± 35.75^{d}	17.65 ± 17.09^{a}

IMI: average number of insects in trap after initial monitoring; ITR: insect-trap ratio; DIT: Insects Mortality in trees, [¶]Mixed with 1 L ha^{-1} of Bionex® adjuvant, [¥]Values with the same letter within each column are not significantly different ($p<0.05$).

(TRP) (incidence reduction: 50%) and red spider mite (RSM) (incidence reduction: 85%), while for the Asian citrus psyllid (ACP), the BM and fruit fly (FF) were ranked as the second-best options, with incidence reductions of 64%, 62%, and 65%, respectively (Table 6 and Figure 1). In this regard, Soto *et al.* (2013) highlighted the effectiveness of sulfur-calcium broth in controlling red spider mite (RSM) populations at concentrations above 20%. Meanwhile, Monteon-Ojeda *et al.* (2020) reported a reduction in TRP populations in mango cultivation of up to 85%.

The results of the repellency effect of Treatment 8 (Control 2, Bannen 1.8%: abamectin) in the present study indicate a possible development of resistance by pests to this chemical, maintaining its effectiveness only for the Asian citrus psyllid (PAC) (incidence reduction greater than 80%) and a partial effect in controlling spider mites (AR) (72% reduction). In this sense, the limited pest control of thrips (TRP), whitefly (WF), and fruit fly (FF), with incidence reductions of approximately 40%, highlights the loss of repellency effectiveness of this chemical against two of the most common pests based on the results obtained during the initial monitoring and the development of this study (Table 6). In this regard, Aguilar *et al.* (2017) determined that the application of abamectin in onion crops showed insecticidal effects with few or no repellency effects.

On the other hand, the repellent effect specifically achieved by the bioinsecticide Biocanela (BC) is also considered suitable for controlling trips (TRP) and red spider mites (AR), with reductions of 48% and 66%, respectively. For whitefly (WF) and fruit fly (FF), treatment 5 (sulfur-calcium broth, SC) demonstrated the best effectiveness as a repellent, with incidence reductions ranging from 74% to 77%. In this regard, Barrón-Contreras *et al.* (2022) determined that, following the application of SC in chili crops, the population of MB is reduced by up to 45%.

The presence of Insects Mortality on the tree (DIT) indicated that treatments based on simple sulfur or in combination with BC (T5: SC and T4: SC+BC), as well as those based on copper combined with Biocanela (T6: CBR+BC), showed the highest insecticidal effect among the agroecological treatments, with a DIT ranging between 11 and 13 insects after treatment (Table 5). Maintaining its residual insecticidal effect for up to 13 days on

Table 6. Average number of insects trapped per pest after the first and fifth applications of each treatment.

Treatments	THRIPS		DIAPHO		BEMISIA		DROSOPH		TETRANYC	
	AP1¥	AP5¥)	AP1¥	AP5¥	AP1¥	AP5¥	AP1¥	AP5¥	AP1¥	AP5¥
T1 Water (absolute control)	9.60	10.47	19.47	18.93	64.13	53.53	52.33	37.93	1.20	1.00
T2 Funlate 50% (benomyl) control sample 1	7.13	9.53	7.00	10.47	40.07	35.00	27.27	24.73	1.00	1.13
T3 Bio-canela	3.27	1.67	7.60	2.73	38.60	18.53	33.07	13.33	1.20	0.40
T4 Sulphocalcium broth + Bio-canela	2.00	1.47	11.67	4.13	39.80	15.07	33.00	11.33	1.07	0.33
T5 Sulphocalcium broth	1.33	1.73	11.67	6.60	56.07	14.27	47.33	10.53	1.20	0.73
T6 Bordeaux broth + Bio-canela	1.73	0.87	7.53	4.07	32.93	14.93	29.60	12.13	3.80	0.53
T7 Bordeaux broth	2.27	1.13	8.13	4.67	42.67	23.00	37.13	17.00	0.80	0.80
T8 Bannen 1.8% (abamectin) control sample 2	2.33	1.40	4.67	0.80	43.60	25.13	34.07	20.27	0.60	0.13

THRIPS: Citrus thrips (*P. kellyanus*); DIAPHO: Asian citrus psyllid (*D. citri*); BEMISIA: Whitefly (*B. tabaci*); DROSOPH: Fruit fly (*D. melanogaster*); TETRANYC: Citrus red spider (*T. urticae*). AP1¥: application 1; AP5¥: application 5.

Figure 1. Insect presence in trees treated with: Bordeaux mixture + Biocanela, a) first count after the first application and b) fifth count after the fifth application; sulfur-calcium broth + Biocanela, c) first count after the first application and d) fifth count after the fifth application.

TRP, PAC, and MB, in the case of treatments including SC (T4 and T5). Martínez (2023) identified the insecticidal effect of cinnamon extract in controlling MB in zucchini crops under open sky conditions, achieving population reductions exceeding 85%.

For the case of Testigo 2 (T8, Bannen 1.8%: abamectin), unlike the repellent results obtained, the insecticidal effect of this treatment was the highest among all the compounds used in the study, exceeding the results obtained by the other treatments by up to 26%, indicating its effectiveness as an insecticide for the pests monitored in the study. In this regard, Restrepo-García and Soto-Giraldo (2017) determined an insecticidal effectiveness of up to 53% against PAC.

Table 7. Accumulated incidence (AI) of fungal diseases identified in the monitoring of Mexican lime trees for each treatment application cycle.

Disease	N	Initial ¶	AP1¥	AP2¥	AP3¥	AP4¥	AP5¥
Anthracnose	120	65.83	65.83	55.83	47.50	37.50	25.00
Sooty mold	120	95.00	95.00	95.00	56.67	30.83	26.67
Algal spot	120	98.33	98.33	95.83	63.33	34.17	24.17
Greasy spot	120	67.50	67.50	67.50	64.17	40.00	29.17

¶Initial incidence value of the fungal diseases in initial monitoring. ¥Accumulated incidence value of the fungal diseases identified in each application. AP1: application 1; AP2: application 2; AP3: application 3; AP4: application 4; AP5: application 5.

Regarding the control of fungal diseases present during the initial monitoring, treatments containing copper (T6: BM with Biocanela; T7: BM, CBR) proved to be the best options for controlling anthracnose, reducing the presence of diseased tissues by 87% to 100% (Table 8). In this context, Park *et al.* (2014) demonstrated a reduction in the presence of anthracnose by up to 61% in bell pepper crops sprayed with BM at 14-day intervals. Similarly, Treatment 6 (BM+BC) shows similar effectiveness in controlling sooty mold, red alga, and greasy spot, being even more effective than the chemical in Test 1 (Funlate 50%: benomyl), which achieved approximately 80% reduction in incidents of anthracnose, sooty mold, and red alga, similar to what was indicated by Guillén *et al.* (2018) for anthracnose and greasy spot in Valencia orange (*Citrus sinensis*) crops. The lower fungicidal effect of benomyl compared to treatments based on mineral broths may indicate the development of probable resistance of field strains to commercial chemical fungicides, as determined by Gutiérrez *et al.* (2003) in mango trees from five regions of the country, where the strains exhibited a median lethal concentration higher than 50 ppm of benomyl.

Although the cumulative incidences of fungal phytopathologies were reduced with the use of copper treatments, a marked defoliation of the trees subjected to these treatments was recorded during the study, which could suggest an episode of copper phytotoxicity, as indicated by Sáenz *et al.* (2019) in citrus subjected to antifungal treatment with copper salts. In this regard, treatment 4 (SCB+BC) also showed significant effectiveness in the control of sooty mold, red algae, and greasy spot (Figure 2).

Similarly to copper compounds, this treatment (SCB+BC) was even more effective in managing some of the reported fungal diseases (sooty mold and red algae). For the control of greasy spot, the efficacy was similar to that determined for Treatment 2 (Funlate 50%: benomyl), where an approximate reduction of 80% and a final accumulated incidence of 2.67% were achieved (Table 8 and Figure 2). This is similar to what was reported by Guillén *et al.* (2018), where they determined that only 1.3% of the trees showed signs of the pathology after treating the crop with benomyl.

On the other hand, although the combined treatment based on sulfur and BC showed antifungal efficacy, the treatments of each compound separately (T3: BC; T5: CS) exhibited limited effectiveness in treating fungal diseases. This is different from what was

Table 8. Effect of the treatments on the Accumulated incidence (AI) of fungal diseases identified in the monitoring of Mexican lime trees at the beginning and end of the study.

Treatments	N	Anthracnose		Sooty mold		Algal spot		Greasy spot	
		Initial	Last	Initial	Last	Initial	Last	Initial	Last
T1 Water (absolute control)	75	17.33	14.67	17.33	17.33	14.67	14.67	13.33	12.00
T2 Funlate 50% (benomyl) control sample 1	75	16.00	2.67	24.200	5.33	14.67	2.67	14.67	2.67
T3 Bio-canela	75	10.67	2.67	17.33	1.33	24.00	2.67	10.67	4.00
T4 Sulphocalcium broth + Bio-canela	75	13.33	5.33	22.67	0.00	24.00	1.33	13.33	2.67
T5 Sulphocalcium broth	75	12.00	2.67	18.67	1.33	21.33	1.33	16.00	4.00
T6 Bordeaux broth + Bio-canela	75	13.33	0.00	21.33	0.00	22.67	0.00	14.67	4.00
T7 Bordeaux broth	75	10.67	1.33	14.67	1.33	20.00	1.33	13.33	4.00
T8 Bannen 1.8% (abamectin) control sample 2	75	12.00	10.67	16.00	16.00	16.00	14.67	12.00	12.00

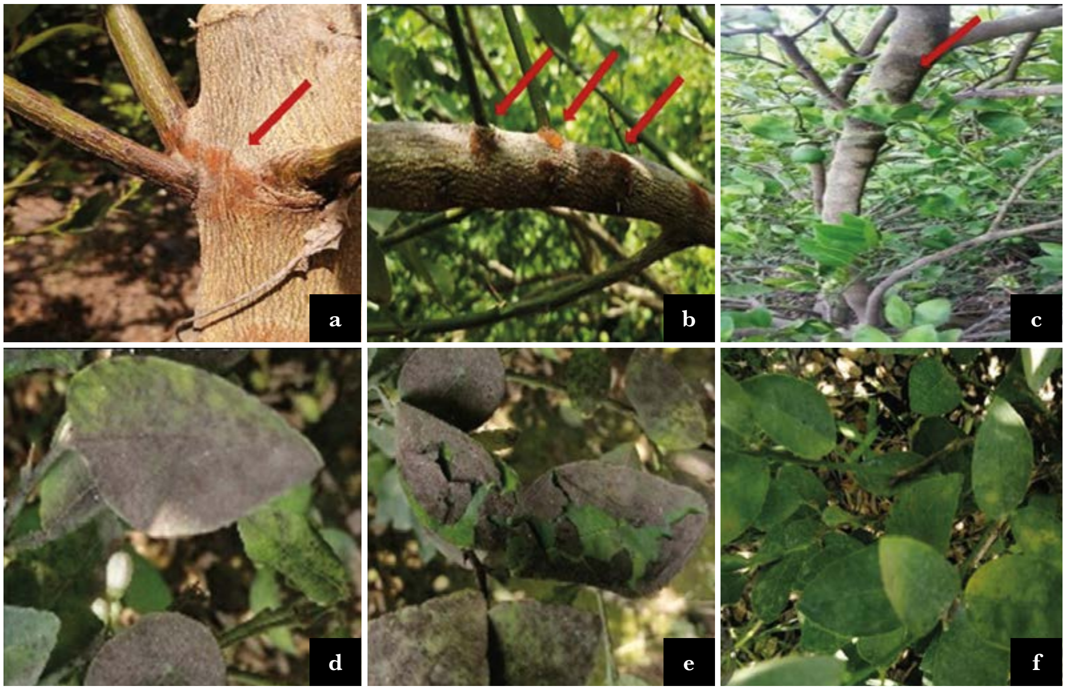

Figure 2. Monitoring of diseases in Mexican lime trees under treatment with Sulfur-Calcium Broth + Biocanela. a) Presence of red algae, initial monitoring. b) 1^{st} count, second application, red algae. c) 3^{rd} count, third application, dry red algae. d) Initial monitoring of diseases, presence of sooty mold. e) 1^{st} count, second application, sooty mold beginning to dry. f) 2^{nd} count, third application, dry and absent sooty mold on the leaf.

determined by Darmadi *et al.* (2022), who found inhibition of anthracnose using crude cinnamon leaf extract.

CONCLUSIONS

Given the overall effectiveness as a repellent, insecticide, and fungicide demonstrated by Treatment 4, the use and application of the combination of sulfur-calcium broth + Biocanela emerges as a viable agroecological alternative for the treatment and control of pests and fungal diseases in Mexican lime crops. On the other hand, the defoliation caused by the use of Bordeaux mixture, both individually and in combination, necessitates a review of dosages and application timing in citrus crops to avoid potential phytotoxicity. Similarly, the results of both chemical controls (T2: benomyl and T8: abamectin), which showed evident limitations against certain pests and fungal diseases, highlight the need for future studies related to possible resistance to these agrochemicals.

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Relationship Between Neutral Detergent Fiber and *In Vitro* Digestibility in Test Crosses of Maize Hybrids

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ABSTRACT

Objective: To relate the physio-technical parameters with the proportions of neutral detergent fiber (NDF) and *in vitro* dry matter disappearance (IVDMD) of test crosses of maize.

Design/methodology/approach: Crosses of early, intermediate, and late maize lines (total=75) from high valleys and subtropical/tropical testers were sowed in three regions of Central-North Mexico. Male and female flowering days (MFD, FFD); days to harvest (Dcor); plant and cob length (PL, CL); forage, corn stover (CS), and cobs humid base (HB) and dry matter (DM) yields; and NDF, acid detergent fiber (ADF), hemicellulose (Hem), crude protein (CP), and IVDMD were analyzed.

Results: High valleys lines had more MFD, FFD, Dcor, and PL, therefore better forage, MS, and cob yields. More MDF, FFD, and H were related to better HB and DM yields, and CP content, but also were related to more NDF, ADF, and Hem proportions, and therefore to a less IVDMD (r=0.47 to 0.98); however, crosslines with high cob yields also had high CS yields (r=0.57-0.68). Regression linear models showed that one unit of NDF might reduce 0.49 to 56% the IVDMD (R^2=0.59-0.78); additionally, NDF<68% was related to IVDMD>60% (R^2=0.63-0.78).

Limitations on study/implications: ADF correlated negatively with IVDMD in early lines; NDF composition should also be related to its degradability (NDFD).

Findings/conclusions: Maize-breeding might be directed to obtain hybrids with less NDF CS contents to use them in ruminant diets, maintaining the cob yields for human nutrition and resistance to plant lodging.

Keywords: Tested maize crosslines (*Zea mays* L.), linear modeling, neutral detergent fiber, dry matter digestibility, corn stover as ruminant feedstuff.

INTRODUCTION

According to the FAO (2019), maize (*Zea mays* L.) cultivation is a global priority. Maize cultivation and consumption impact the social, environmental, and economic conditions in Mexico (Domínguez *et al.*, 2018; Ibarrola *et al.*, 2020). Consequently, along with the

priority of funding social and environmental projects (IATP, 2022), public policies and actions are being implemented to ensure its production (Castro and Montpetit, 2017). Numerous previous studies have addressed objectives related to the effects of variations in agronomic management practices and the identification of maize hybrids to increase grain yield and composition (García-Lara *et al.*, 2020; Sánchez *et al.*, 2022). Additionally, alternatives have been sought to ensure the sustainability of maize cultivation, addressing not only the economic impact but also the environmental and social dimensions (Gayosso-Barragán *et al.*, 2021, 2023; FAO, 2024) in areas where producers are vulnerable due to reduced availability of productive land and water resources (Ibarrola *et al.*, 2020; Chávez-Aguilar *et al.*, 2023).

Genetic improvement can be directed towards increasing the content and quality of crude protein (CP), soluble and insoluble fibers, oils, sugars, and starch, as well as carotenoids and flavonoids in the grains (Yang and Zhai, 2010a, 2010b; Salinas-Moreno *et al.*, 2012, 2013). These outcomes contribute to enhancing the nutritional status of the majority of Mexicans (Vivek *et al.*, 2008). Other objectives of genetic improvement, such as increasing resistance to water stress, pests, or diseases, enhancing the earliness of varieties, and reducing lodging incidence, are crucial for arid or semi-arid regions, as they lead to better management of inputs and a greater margin between costs and profits (Medina-Cuéllar *et al.*, 2021). However, improving these characteristics can affect the composition of the stalks and leaves (corn stover (CS)) in terms of their cell wall proportion (primarily quantified by Neutral Detergent Fiber (NDF)) and the proportions of hemicellulose, cellulose, lignin, and CP, which will impact the ruminal digestibility of Dry Matter (DDM) and, therefore, the feasibility of using the forage (ears and CS) for ruminant feed (Tirado-Estrada *et al.*, 2021).

Improving the yield and DMD of CS as an alternative for animal feed could be a strategy that reduces the economic and environmental costs of animal production. Historically, genetic improvement programs and crop management have focused on maximizing grain production and quality while also improving CS composition (Peña *et al.*, 2006; Stendal *et al.*, 2006; Staton *et al.*, 2007; González-Manzano *et al.*, 2008). Specifically, studies have demonstrated how modifications in the structure and chemical composition of NDF can affect its degradability (Jung and Casler, 2006a, b) and, in turn, how increasing ruminal digestibility of NDF (DFDN) can promote reduced grain use in feed without compromising animal production potential (Oba and Allen, 1999). Increasing the DFDN helps improve animal productivity, reduce production costs, and lessen the environmental impact resulting from deforestation needed to maintain intensive grain production. Variations in the proportion and composition of NDF can be attributed to biological cycles, germplasm origin, and various environmental stresses (Tirado-Estrada *et al.*, 2021). Therefore, modeling can be useful in establishing the relationship between these factors, which affect the utilization of DM and, consequently, animal productive performance (Miranda-Romero *et al.*, 2020). In this study, linear models have been used to establish the relationship between physio-technical and performance variables, with the proportion of NDF and *in vitro* digestibility of DM (IVDMD) of early/intermediate/late maize crosses of subtropical origin and high valleys.

MATERIALS AND METHODS

Location

This study was conducted at the facilities of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) located in Pabellón de Arteaga, Aguascalientes, Mexico (102° 26' W, 22° 09' N, 1900 masl, average annual temperature (T) of 18 °C, 440 mm precipitation/year, xerosol and regosol soils); Torreón, Coahuila, Mexico (26° 44' N, 105° 10' W, 1510 masl, T of 14 °C, 501 mm precipitation/year, luvisol xerosol soils); and Las Delicias, Chihuahua, Mexico (28° 25' N, 105° 02' W, 1145 m asl, T of 18.3 °C, 334 mm precipitation/year, eutric regosols).

Genetic Material

Early, intermediate, and late S2 crosses with low endogamy, derived from simple crosses of testers provided by CIMMYT, INIFAP, and commercial sources, adapted to altitudes of 1600 to 2200 meters above sea level or to subtropical/tropical regions, were analyzed. The description and evaluation of the testers and test crosses were detailed by Peña *et al.* (2004).

Agronomic Management and Sampling

Total areas of 1 hectare (150 plots) were planted, blocked according to orientation and slope. Planting was conducted under irrigation conditions, with a density of 80,000 plants/ha. Fertilization was done using N-P-K doses of 200-90-00 kg/ha, applying half of the nitrogen and all of the phosphorus during the first weeding (between 25 to 30 days after planting) and the remaining nitrogen was applied between 25 and 30 days after the first weeding. Three supplementary irrigations were given to the group of early hybrids and four to the intermediate and late ones. Additionally, the corresponding agronomic management for maize cultivation was carried out in each region, including integrated pest, disease, and weed management. When the maize grains reached 58 to 60% dry matter (DM), a sample of 25 plants was randomly harvested from each experimental plot. The whole plants and ears were chopped and mixed, considering a particle size according to the method reported by Krause and Combs (2003).

Evaluated Variables

Data were collected on physio-technical variables: days to male flowering (DMF) and female flowering (DFF), days to harvest (Dcor), plant height (PH), and ear height (EH). During sampling, the fresh weights (FW) of the following were recorded: whole plants (Forage), stalks, leaves, and husks (corn stover (CS) and ears (cobs and grains)). Samples of 1 kg of whole plants, corn stover (CS), and ears were collected and placed in a forced-air oven at 60 °C until they reached a constant weight (DM) (González *et al.*, 2005). Subsequently, the dry samples of whole plants were ground using a Thomas-Willey mill (Laboratorios Mill) with a 1 mm screen. The proportions of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (Hem), and *in vitro* digestibility of dry matter (IVDMD) were determined. Based on the fresh and dry matter weights of corn stover (CS) and ears, the proportions of CS (%CS=kg of CS/kg of forage) and ears (%Ears=kg of ears/kg of forage) were calculated.

Statistical Analysis

The SAS statistical package (Statistical Analysis System, V. 9.4; 2013) was used to perform analysis of variance (ANOVA) employing general linear or mixed procedures (Proc GLM, Proc Mixed), considering random effects of lines within locations and fixed effects of biological cycle and germplasm origin, according to Model 1. Adjusted means (LSMeans), coefficients of determination, and variation (R^2 and CV) are reported.

$$Y_i = L(Loc)_{ij} + Cycle_k + Origin_l + (Cycle * Origin)_{kl} + \varepsilon_{ijkl} \qquad \text{Model (1)}$$

Where: Y_i=DFF, DMF, PH, EH; fresh and dry matter yields of forage, corn stover (CS), and ears; %DM; CP, IVDMD, NDF, ADF, Hem; $L(Loc)_{ij}$ =random effect of the *i*-th line within the *j*-th location; $Cicle_k$=fixed effect of the *k*-th biological cycle; $Origen_l$=fixed effect of the *l*-th germplasm origin; $(Cycle * Origin)_{kl}$ =interaction between factors; ε_{ijkl}=random error.

Correlation and Multiple Linear Regression Analysis

The simple relationships between the evaluated variables were analyzed by calculating Pearson correlations (Proc Corr). Simple linear regression models were obtained using the Stepwise (Forward) procedures (Proc Reg), including variables with $P<0.15$ and considering R^2 and Mallows' Cp as criteria for validity.

RESULTS

Table 1 shows the physiotechnical variables evaluated in the crop. The days to female flowering (DFF) and male flowering (DMF) were greater in late crosses ($P<0.0001$). However, more days were required for male flowering (DMF) and for harvesting (DCor) in lines from high valley testers for intermediate and late crosses ($P<0.01$). Plant height (PH)

Table 1. Physiotechnical variables evaluated in 75 maize hybrid test crosses.

Phenological cycle	Origin	FFD (d)	MFD (d)	DCor (d)	PH (cm)	EH (cm)
Early	High valleys	69.18	64.73	117.07	241.71	109.05
	Subtropical/Tropical	68.49	65.22	117.66	233.81	100.95
Intermediate	High valleys	77.15	73.43	124.94	246.88	117
	Subtropical/Tropical	76.60	71.72	123.38	245.7	112.95
Late	High valleys	80.41	76.68	129.76	260.2	114.71
	Subtropical/Tropical	79.94	75.11	128.68	248.23	111.56
R^2		0.88	0.92	0.95	0.72	0.35
CV (%)		3.53	3.15	2.44	6.49	13.56
Cycle		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Origin		0.51	0.11	0.36	0.16	0.03
C*O		0.97	<0.0001	0.01	0.03	0.53

DFF, days to female flowering; DMF, days to male flowering; DCor, days to harvesting; PH, plant height; EH, ear height; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

and ear height (EH) increased with the biological cycle and were generally higher in lines from high valleys (P<0.03).

In Table 2, the humid base and dry matter yields of forage, corn stover (CS), and ears (ear without husks) were better in lines from high valleys compared to those from subtropical/tropical origins (P<0.002). However, although early-cycle lines had lower yields in humid base and dry matter, intermediate-cycle lines had better forage, CS, and ear production than late-cycle lines (P<0.005). The proportions of ears relative to total forage ranged from 30.37% to 34.45% in humid base and between 47.46% and 52.82% in dry matter. However, the proportion in humid base was better in late hybrids, while in dry matter, it was better in early hybrids (P<0.02).

Table 3 shows that there were differences between lines of different biological cycles in terms of dry matter (DM), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD) proportions, as well as in the composition of neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (Hem). Although intermediate-cycle lines had higher CP contents, early-cycle lines exhibited better IVDMD and lower proportions of NDF, ADF, and Hem (P<0.002). IVDMD was higher in lines from subtropical/tropical testers compared to lines from high valleys testers (P<0.001).

Lines from high-valley testers had higher FFD, MFD, DCor, and plant height (PH) than those from subtropical/tropical origins. Additionally, they showed better yields of forage, CS and Ear in both humid base (HB) and dry matter (DM). Late varieties had lower yields compared to intermediate crosses. Early crosses and those from subtropical/tropical testers had lower contents of NDF, ADF, and Hem and better IVDMD. Intermediate lines had higher CP content compared to the other crosses. Longer biological cycles may favor better

Table 2. Humid base (HB) and dry matter (DM) yields in 75 maize hybrid test crosses.

Phenological cycle	**Origin**	**Wet yield (kg WB/ha)**						**DM Yield (kg DM/ha)**			
		Forage	**Stover**	**Cob**	**%S**	**%Cob**	**Forage**	**Stover**	**Cob**	**%S**	**%Cob**
Early	High valleys	69824	48320	21504	69.63	30.37	25952	13344	12576	51.67	48.33
	Subtropical/ Tropical	62464	41152	21312	65.58	34.42	24192	11424	12736	47.18	52.82
Intermediate	High valleys	79648	53984	25632	67.79	32.21	30912	16096	14816	51.83	48.17
	Subtropical/ Tropical	68512	46688	21760	68.2	31.80	26208	13728	12448	52.52	47.48
Late	High valleys	72800	48000	24736	65.63	34.37	29472	15392	14048	52.54	47.46
	Subtropical/ Tropical	64736	43840	20896	67.08	32.92	25664	13440	12256	52.39	47.61
R^2		0.71	0.64	0.71	0.33	0.33	0.48	0.38	0.55	0.42	0.42
CV (%)		15.72	18.66	16.33	6.3	6.3	15.18	20.86	17	12.46	12.46
Cycle		<0.0001	<0.0001	<0.0001	0.02	0.02	<0.0001	<0.0001	0.005	<0.0001	<0.0001
Origin		0.002	0.005	0.006	0.48	0.48	0.001	0.005	0.02	0.46	0.46
C*O		0.32	0.33	<0.0001	<0.0001	<0.0001	0.008	0.79	<0.0001	0.001	0.001

CS, corn stover; Forage, whole plants; CS, corn stover; Cob, cobs without husks; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

Table 3. Dry matter (DM) proportion, *in vitro* digestibility, and composition of test crosses of early/intermediate/late lines from high valley or subtropical/tropical testers.

Phenological cycle	Origin	DM (g/100 of WB yield)			Composición (g/100 MS)				
		Forage	Stover	Cob	CP	IVDMD	NDF	Hem	ADF
Early	High valleys	37.62	28.04	60.39	8.00	71.83	56.85	27.35	29.51
	Subtropical/Tropical	39.70	28.61	60.34	8.37	74.04	55.68	27.69	27.98
Intermediate	High valleys	39.99	31.15	59.09	8.57	69.19	60.78	29.51	31.27
	Subtropical/Tropical	39.36	30.49	58.15	8.51	72.74	59.47	30.75	28.73
Late	High valleys	43.33	34.91	58.82	8.06	66.83	60.16	28.56	31.6
	Subtropical/Tropical	41.73	33.05	59.46	8.37	70.24	61.3	30.91	30.39
R^2		0.60	0.58	0.63	0.89	0.43	0.31	0.33	0.28
CV (%)		12.63	19.27	7.4	8.29	4.04	7.91	9.82	11.28
Cycle		<0.0001	<0.0001	0.002	0.006	<0.0001	<0.0001	<0.0001	<0.0001
Origin		0.99	0.73	0.82	0.45	0.001	0.66	0.20	0.09
C*O		0.02	0.31	0.4	0.09	0.23	0.22	0.11	0.42

*HB, humid base; DM, dry matter; total, whole plant; CS, corn stover; Cob, cobs without husks; CP, crude protein; IVDMD, *in vitro* dry matter digestibility; NDF, neutral detergent fiber; Hem, hemicellulose; ADF, acid detergent fiber; R^2, coefficient of determination; CV, coefficient of variation; C*O, cycle*origin interaction.

humid base (HB) and dry matter (DM) yields and higher crude protein (CP) content but negatively impact in vitro dry matter digestibility (IVDMD) by increasing the amount of neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (Hem) (r=0.47 to 0.98; P<0.0001). However, varieties that produce a higher amount of grain could also be useful in terms of corn stover (CS) yield (r=0.57 to 0.68; P<0.0001).

NDF contributes negatively by 63 to 78% to IVDMD in early crosses, from high valley testers, and from subtropical/tropical origins (R^2=0.63 to 0.78; P<0.0001) (Table 4). In general, to achieve an IVDMD of >60 g/100 DM, the NDF should be less than 68%. In early and late crosses, and those from testers adapted to high valleys or from subtropical/tropical origins, the proportion of NDF is negatively related by 59 to 78% to IVDMD. According to these models, an increase of one unit of NDF would result in a reduction of 0.49 to 0.56% in IVDMD (R^2=0.59-0.78; P<0.0001). However, in some lines, such as intermediate ones, a unit increase in ADF can reduce IVDMD by up to 0.66% (R^2=0.70; P<0.0001). Other variables such as MFD, DCor, or the proportions of CS or cob may be related to IVDMD; these contribute less to the R^2 of the models (4 to 7%).

DISCUSSION

In this study, we found that later maturing hybrids might increase the production of DM from forage, stover, and ears. However, they can also reduce in vitro digestibility of DM (IVDMD) by favoring the increase of neutral detergent fiber (NDF) and acid detergent fiber (ADF); this is possible because as the maize maturity period extends, the proportion and composition of lignin and the types of bonds linking it with some hemicellulose structures change, making NDF a less digestible compound (Jung and Casler, 2006a, b). Nevertheless, the intermediate hybrids evaluated not only showed

Table 4. Multiple Linear Regression Models for Neutral Detergent Fiber (NDF) and *In Vitro* Dry Matter Digestibility (IVDMD).

$Y_i=NDF$	$Y_i=\beta_0+X_i\beta_i+\varepsilon_{ij}$	R^2
Early	$Y_i=136.10-1.13\ IVDMD+\ _{ij}$	0.63
Intermediate	$Y_i=26.34+1.20\ ADF+\varepsilon_{ij}$	0.71
Late	$Y_i=30.22+1.03\ ADF+\varepsilon_{ij}$	0.60
High valleys	$Y_i=156.85-1.40\ IVDMD+\varepsilon_{ij}$	0.78
Subtropical/Tropical	$Y_i=167.64-1.54\ IVDMD+\varepsilon_{ij}$	0.70
	$Y_i=110.27-1.05\ IVDMD+0.75\ Hem+\varepsilon_{ij}$	0.88
$Y_i=IVDMD$		R^2
Early	$Y_i=102.58\text{-}0.55\ NDF+\varepsilon_{ij}$	0.63
	$Y_i=112.8\text{-}0.48\ NDF+-0.21\ MFD+\varepsilon_{ij}$	0.69
Intermediate	$Y_i=88.96-0.66\ ADF+\varepsilon_{ij}$	0.70
	$Y_i=93.52-0.46\ ADF-0.17\ NDF+\varepsilon_{ij}$	0.73
	$Y_i=117.11-0.40\ ADF-0.24\ NDF-0.15\ HT+\varepsilon_{ij}$	0.77
Late	$Y_i=98.30-0.49\ NDF+\varepsilon_{ij}$	0.59
	$Y_i=102.24-0.49\ NDF-0.2\ Stover\ WB+\varepsilon_{ij}$	0.65
High valleys	$Y_i=103.19-0.56\ DNF+\varepsilon_{ij}$	0.78
	$Y_i=105.43-0.50\ NDF-0.63\ Cob\ DM+\varepsilon_{ij}$	0.84
Subtropical/tropical	$Y_i=97.12-0.45\ NDF+\varepsilon_{ij}$	0.70
	$Y_i=97.45-0.31\ NDF-0.31\ ADF+\varepsilon_{ij}$	0.75
	$Y_i=108.68-0.23\ NDF-0.36\ ADF-0.11\ HT+\varepsilon_{ij}$	0.79

ADF, acid detergent fiber; Dcor, days to harvest; DM ear, ear yield on a humid base; DM, dry matter; CS, corn stover; BH, humid base; R^2, coefficient of determination.

better proportions of crude protein (CP) but, in some cases, also better yields of forage, stover, and ears, and even better *in vitro* digestibility of DM (IVDMD) than the late varieties. This demonstrates that it is possible to direct maize genetic selection towards obtaining earlier hybrids with similar yields of ears and stover (Medina-Cuéllar *et al.*, 2021) without reducing their IVDMD.

According to Van Soest *et al.* (1991), the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF) allows for the differentiation of some basic cell structures, such as the total content of cell walls (NDF) and the combined content of cellulose, lignin, tannins, and silicates (ADF). In line with the findings of the present study, the selection of hybrids with better nutritional characteristics for the formulation of ruminant diets has been directed towards hybrids with lower contents of NDF (Stendal *et al.*, 2006; Staton *et al.*, 2007).

In previous studies, we found that, in addition to the proportion of NDF, it is important to differentiate the contents of cellulose, hemicellulose, and lignin, which can vary depending on the germplasm origin. Some of the hybrids generated in the present study, originating from highland testers, may contain more lignin than hybrids from subtropical or tropical testers, affecting the *in vitro* digestibility of DM (DIVDM) even though they had lower

NDF contents (Tirado-González *et al.*, 2016). Several studies conducted by Oba and Allen (1999, 2000a, b, c, 2003) demonstrated that, in addition to the NDF content, its individual digestibility should also be considered. They found that NDF digestibility (NDFD) is directly related to the potential for milk production (corrected to 4% fat). At the ruminal level, small variations in the composition of NDF can modify ruminal fermentation and the potential for fiber utilization (Soufizadeh *et al.*, 2018; Miranda-Romero *et al.*, 2020). This means that, in addition to composition, variations in the quantity and types of bonds between hemicellulose and lignin modify the ruminal potential for obtaining energy from cellulose and hemicellulose (Tirado-González *et al.*, 2018, 2021; Carrillo-Díaz *et al.*, 2022). These aspects have been considered relevant for increasing the proportion of fiber in ruminant diets (Soufizadeh *et al.*, 2018; Tirado-Estrada *et al.*, 2021). Furthermore, seeking the inclusion of higher proportions of CS without reducing the productive potential of ruminants is a strategy that can promote the sustainability of meat and milk production, considering the reduction of economic and environmental costs (Adesogan *et al.*, 2019; Tirado-Estrada *et al.*, 2021; FAO, 2024).

CONCLUSIONS

The simple regression models reported in this study show that for hybrids from testers of highland or subtropical/tropical germplasm origins, an increase of one unit of NDF can reduce IVDDM by between 0.49% and 0.56%. To ensure an IVDDM greater than 60%, NDF must be less than 68%. Although the results of these models should be validated in the future, considering their R^2 values, we can assume that at least in Mexican lines from testers of highland or subtropical/tropical origins, reducing NDF may be a viable strategy to improve the IVDDM of CS. The results found in this study may be helpful in identifying maize hybrids whose CS can be efficiently used in ruminant feeding.

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Colegio de Postgraduados

Selection of Advanced Bread Wheat Lines for Their Response to Premature Ripening Caused by *Fusarium* sp.

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ABSTRACT

Objective: Screen a bread wheat trial for resistance to Crown root rot.

Design/methodology/approach: one hundred and fifty experimental wheat lines with different genetic and physiological characteristics, were inoculated under greenhouse conditions with a mixture of five species of Fusarium, which were isolated from wheat commercial fields. The response of the wheat germplasm to the disease was scored through a disease index.

Results: Sixty-three bread wheat experimental lines were identified with a disease severity of 1 to 9%, which are considered as resistant.

Limitations on study/implications: The evaluation of wheat germplasm for disease resistance in the field presents important variables, which cannot be controlled, mainly for the spatial distribution of pathogens; hence, a better option is to carry out the trials under a controlled environment.

Findings/conclusions: Eleven genotypes were selected based on their resistance to Crown rot and to a better grain yield per spike.

Keywords: Wheat, Crown root rot, *Fusarium* sp., Genetic resistance.

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INTRODUCTION

Wheat is one of the most important cereals in human nutrition, along with maize, rice, and potatoes. One of the main diseases affecting wheat worldwide is caused by several species of the genus Fusarium, known as Wheat Premature Ripening (WPR), also referred to as Drying or Crown Rot Root. This disease has been reported in Australia, China, the USA, Argentina, India, Ukraine, Western Asia, Northern Africa, South America, and Mexico (Cook, 2010; Ôzdemir, 2022; Petronaitis *et al.*, 2024; Mariscal *et al.*, 2018; Wildermuth *et al.*, 1997). The complex of *Fusarium* species is of utmost importance in wheat cultivation, as it can cause yield losses of up to 89% (Klein *et al.*, 1991; Liu and Odbonnaya, 2015). These pathogens develop survival structures (chlamydospores) that can remain viable in the soil for several years. Currently, different *Fusarium* species causing root, crown, and stem rots have been identified. The Bajío region is the most important area for bread wheat production in Mexico, with an approximate cultivation area of 130,000 hectares (SIAP, 2024). The varieties cultivated in this region are of the bread and soft gluten type (Villaseñor-Mir *et al.*, 2022). Wheat Premature Ripening (WPR) or Drying is a disease that occurs in various parts of the Bajío, causing yield losses of 10-60% (Mariscal *et* *al.*, 2018). Root, stem, and crown rots in wheat can occur in all cereal-producing areas and are considered more aggressive in humid climates or environments (Gilchrist-Saavedra *et al.*, 2005). In various etiological studies of the disease conducted in the Bajío, the following species have been identified: *Fusarium proliferatum*, *F. graminearum*, *F. culmorum*, *F. equiseti*, *F. poae*, *F. nivale*, *F. longipes*, *F. subglutinans*, *F. dimerum*, *F. tricintum*, *F. verticillioides*, *F. oxysporum*, *F. thapsinum*, and *F. andiyazi* (Mariscal *et al.*, 2018; Rangel-Castillo *et al.*, 2017; Leyva-Mir *et al.*, 2017; Suaste-Franco *et al.*, 2020). In wheat seedlings, small brown lesions can be observed on the root, crown, and leaf sheaths or on the lower part of the stem. In the adult stage, a coppery wilting of the spikes can be observed in diseased plants. This increases when there is a period of water stress from anthesis to maturity (Liu and Ogbonnaya, 2015; Moya, 2013). In addition to the symptoms of WPR, reduced foliar growth, a decrease in the number of tillers, and lower quantity and quality of grains can also be observed. The best control of wheat diseases is through genetic resistance, which can be selected and derived from experimental germplasm. In the pre-improvement wheat program developed through the collaboration between the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP) and the International Maize and Wheat Improvement Center (CIMMYT), advanced lines tolerant to WPR have been generated as one of the alternatives to reduce the damage caused by the *Fusarium* species complex (Ôzdemir, 2022; Liu and Ogbonnaya, 2015; Suaste-Franco, 2020). Therefore, it is necessary to generate germplasm banks that contain information on the response of experimental wheat lines to WPR, to integrate them into the breeding program with the aim of obtaining lines resistant to this disease that could later be released as commercial varieties. Thus, the objective of this work was to evaluate the reaction of four groups of advanced wheat lines to Premature Ripening caused by *Fusarium* spp.

MATERIALS AND METHODS

Isolation and Production of *Fusarium* sp. Inoculum

Samples of diseased wheat plants were obtained from the localities of La Laja de Cervantes and La Maltaraña, both in the municipality of Jamay, Jalisco. Twenty-two isolations were made from roots, crowns, and stems with infection symptoms. The isolated and purified species were identified based on their morphology and physiology, including type of mycelium, growth rate, colony color and appearance, conidia morphology, and chlamydospores. For inoculum production, the "oat grain colonization" method (Erginbas-Orakci *et al.*, 2016) was used, with only the five most frequent and identified *Fusarium* spp. species. Plastic bags of 15×10×35 cm were employed. A mixture of peat moss, sand, and perlite in a 1:1:1 ratio was used as the substrate.

The inoculum was placed at the midpoint of each pot, where 10 seeds of each experimental wheat line were sown. The trials were conducted in a greenhouse at the Centro Altos de Jalisco Experimental Station of INIFAP, in Tepatitlán, Jalisco. An experiment was designed with four groups of wheat lines and two repetitions (healthy and inoculated). The four groups of wheat lines were: 1) Trial 1R, with 36 entries of bread wheat, sourced from the INIFAP national wheat program and specifically adapted to the Bajío region; 2) Trial LTP-Fusarium, consisting of 45 wheat lines with different agronomic characteristics, from the Seed Discovery initiative of CIMMYT; 3) Trial 10° SATYN (Stress Adapted Trait Yield Nurseries), with 36 advanced bread wheat lines, from a nursery evaluated and selected under extreme heat and/or drought conditions, also from CIMMYT; and 4) Trial 8° WYCYT (Wheat Yield Consortium Yield Trial), with 33 bread wheat lines, from a high-yielding grain nursery of CIMMYT.

Germplasm was assessed for symptom presence at 14 and 70 days after sowing (DAS) using a severity scale from 1 to 5, proposed by Wildemuth *et al.* (1997) and modified by Ôzdemir (2022). The scale was as follows: 1=1-9% as resistant, 2=10-29% moderately resistant, 3=30-69% moderately susceptible, 4=70-89% susceptible, and 5=50-99% highly susceptible. The scale data were transformed using the formula $\sqrt{x+1}$, where x=scale value (Gómez & Gómez, 1984). Grain yield per spike was obtained for each wheat line, and a regression analysis was performed between the incidence of WPR and grain yield.

RESULTS AND DISCUSSION

Twenty strains were isolated, of which 5 species were identified as the most frequent: *Fusarium verticillioides*, *F. andiyazi*, *F. graminearum*, *F. chlamydosporum*, and *F. globosum*, according to Leslie's classification system (2006). The identified species partially match those reported in the Bajío (Mariscal *et al.*, 2018; Rangel-Castillo *et al.*, 2017; Leyva-Mir *et al.*, 2017), although they differ from reports in Australia, Argentina, or the USA, as *F. pseudograminearum* was not found.

Regression analyses of the incidence of Drying at 70 DAS in relation to grain yield per spike revealed that only the lines from the 10° SATYN trial showed a negative effect on yield.

Based on the transformed and graphed values of WPR severity, it is observed that the lines from the Trial 1R are moderately resistant to moderately susceptible, while the other three trials fall within the resistant range (Figure 1).

From each trial, lines with the lowest incidence of WPR and the best grain yield per spike were selected (Table 4).

The group of lines with the highest tolerance was the 8° WYCYT, with 18 lines (54%) in the resistant category and 12 lines in the moderately resistant category, followed by the 10° SATYN trial with 17 lines (47%) in the resistant category; the LTP-Fusarium trial with 16 lines (35%) resistant, and finally, the 1R trial with 12 lines (33%) resistant and 5 lines as moderately susceptible. When selecting by yield, it was observed that lines rated in scales 1 and 2 showed the best grain yields per spike (Table 4). In the 8° WYCYT trial, the lines are characterized by having a higher yield capacity under irrigation conditions. However, for the Bajío conditions, they fall outside the production system due to their longer vegetative cycle, up to 20 days longer (124 days to maturity) compared to the 1R lines, which are the earliest, with an average of 113 days.

The effect of WPR was from slight to moderate, as most of the wheat lines were rated between values 1 and 3 (Table 2), and the regression equations showed little effect. Only in the 10° SATYN trial was a low negative effect on yield observed (Figure 1c).

These results are preliminary and indicate that, while there are lines with tolerance genes, these need to be identified in detail. Field selection of wheat germplasm for

Table 1. Morphological characteristics of *Fusarium* species used in the inoculation of wheat lines.

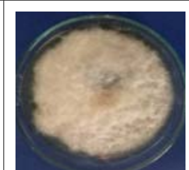
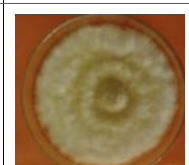
Species	Growth ratio colony	Morphology colony	Pigmentation
F. verticillioides	14.7	Mycelium dowmy White color turning grayish-violet.	
F. andiyazi	16.2	Powdery mycelium, White color turning grayist salmon color	
F. graminearum	20.3	Abundant mycelium, downy White-yellow color, turning red-brown color.	
F. chlamydosporum	10.8	Downy mycelium, White color, turning yellow.	
F. globosum	12.1	Downy mycelium, White color turning violet.	

Figure 1. Characteristic symptoms of WPR under field conditions, from which the *Fusarium* spp. species were isolated.

Table 2. Wheat lines classified by their response to Wheat Premature Ripening at 70 days after sowing, artificially inoculated in pots and under greenhouse conditions.

SCALE (1-5)	Trials of wheat experimental lines			
	LTP-Fusarium	8°WYCYT	1R	10°SATYN
1	16*	18	12	17
2	9	12	0	14
3	14	3	19	3
4	6	0	5	2
5	0	0	0	0
Total:	45	33	36	36

*Number of wheat lines selected in each severity scale.

Table 3. Regression equations and parameters of disease incidence at 70 DAS versus grain yield per spike for each of the wheat trials.

Trials	Equations	R^2	MSE	C.V.
LTP	$Y=4.444+12.592\ X$	0.168	74.51	32.29
8°WYCYT	$Y=30.028+1.257\ X$	0.0009	71.16	26.34
IR	$Y=19.393+7.580\ X$	0.118	43.26	19.73
10°SATYN	$Y=27.406-1.084\ X$	0.0018	38.86	24.31

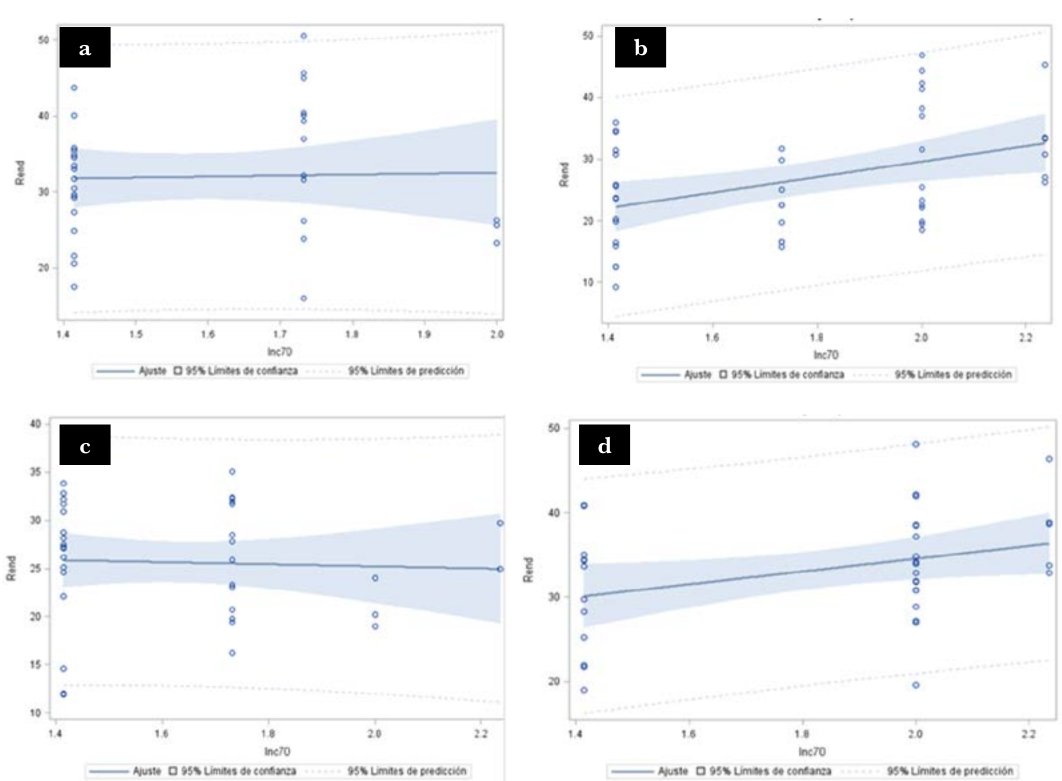


Figure 2. Regression graphs for the trials: a) 8° WYCYT, b) LTP-Fusarium, c) 10° SATYN, and d) Trial 1R.

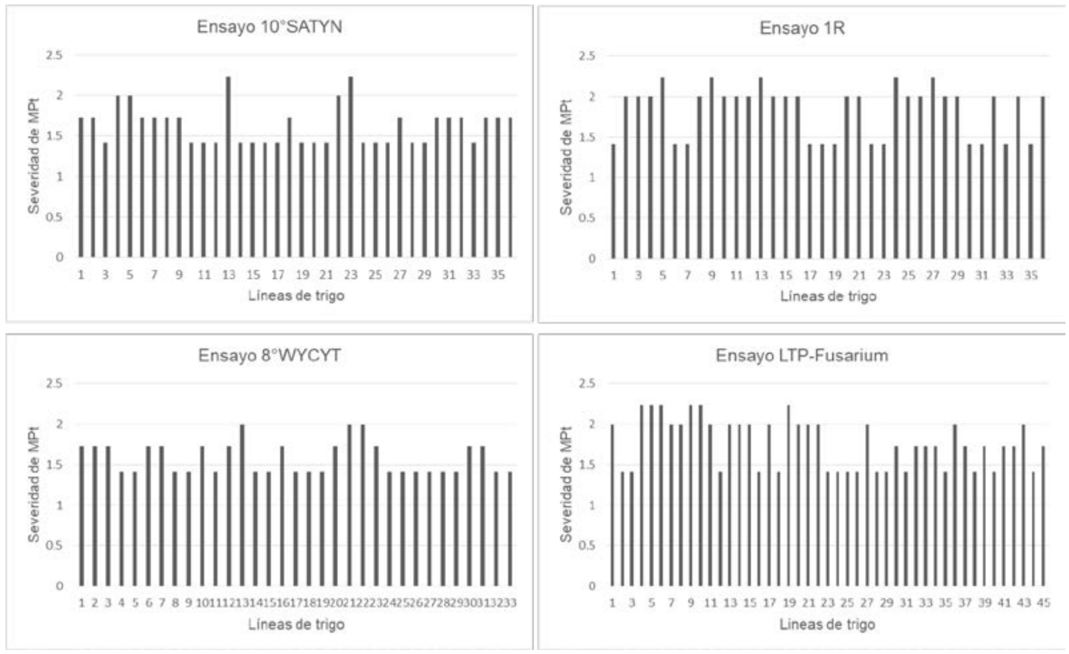


Figure 4. Severity of Wheat Premature Ripening in 150 experimental wheat lines, artificially inoculated with 5 *Fusarium* species under greenhouse conditions.

resistance to *Fusarium* spp. is inefficient due to the irregular distribution of the pathogen. In contrast, selection under greenhouse conditions increases efficiency, although it is not 100%. Therefore, different inoculation methods and disease assessment techniques should be combined (Petronaitis *et al.*, 2024). In the Ciénega de Chapala region, wheat is rotated with maize, and this system has led to an increase in *Fusarium* spp. infections in both wheat and maize. This rotation system may influence the prevalence of certain *Fusarium* spp. species in both crops (Ireta *et al.*, 2023).

CONCLUSIONS

Sixty-three experimental wheat lines were identified as resistant (1 to 9%), 35 lines as moderately resistant (10-29%), 39 lines as moderately susceptible (30-69%), and 13 lines as susceptible (70-89%) to the severity of Wheat Premature Ripening, after being inoculated with the *Fusarium* complex including *F. verticillioides*, *F. andiyazi*, *F. graminearum*, *F. chlamydosporum*, and *F. globosum* under greenhouse conditions.

The best lines based on their tolerance to WPR and grain yield per spike were: H-1539/BORL14/3/FRET22/SHAMA//KACHU in the LTP-Fusarium trial; SO-KOLL/5/W15.92/4/PASTOR//HXL7573/2BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2BAU in the 10° SATYN trial; TUR.180085//QUAIU2/KINDE in the 8° WYCYT trial; and COLI-BRI/ACACIA in the 1R trial.

In selecting wheat germplasm for disease resistance, a combination of disease response and grain yield should be considered. Under this concept, as shown in Table 4, the LTP-Fusarium, 8° WYCYT, and 1R trials had better grain yields compared to the 10° SATYN trial, although all selected lines remained within the 1 to 2 range on the severity scale.

Table 4. Crossing and selection history of the lines selected for their response to WPR incidence and grain yield.

Crossing and Selection	Incidence (Scale 1-5)	Yield (gr spike)
Trial: LTP-Fusarium		
H-1539/BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00156T-0M-0Y-0B-0Y-0B-20Y	2	46.9
CETA/AE.SQUARROSA (299)//BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00144T-0M-0Y-0B-0Y-0B-28Y	2	44.4
D67.2/PARANA 66.270//AE.SQUARROSA (741)/3/BORL14/4/FRET2*2/SHAMA//KACHU SDSS14Y00123T-0M-0Y-0B-0Y-0B-58Y	2	42.4
IG 41643/BORL14/3/FRET2*2/SHAMA//KACHU SDSS14Y00128T-0M-0Y-0B-0Y-0B-52Y	2	41.5
Trial: 10° Satyn		
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-6Y-020Y-0B	2	35.1
SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00138S-099B-099Y-099M-5Y-020Y-0B	1	33.9
KS940935.7.1.2/2*PASTOR/4/FRAME//MILAN/KAUZ/3/PASTOR/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS14Y00103S-0B-099Y-099B-8Y-020Y-0B	1	32.8
SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00138S-099B-099Y-099M-9Y-020Y-0B	2	32.4
MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN/4/PUB94.15.1.12/WBLL1/5/MUCUY PTSS14Y00328S-0B-099Y-099B-19Y-020Y	2	32.3
SOKOLL/WBLL1/4/PIHA//WORRAKATTA/2*PASTOR/3/PRL/2*PASTOR PTSS15Y00152S-099B-099Y-099M-24Y-020Y-0B	1	32.2
BAJ #1 CGSS01Y00134S-099Y-099M-099M-13Y-0B	2	32
SOKOLL/WBLL1/5/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM/4/VORB PTSS15Y00111S-099B-099Y-099M-2Y-020Y-0B	1	31.7
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-25Y-020Y-0B	2	31.7
Trial: 8° Wycyt		
TUR.180085//QUAIU*2/KINDE PTSS16Y00047S-0B-099Y-099M-20Y-0B-0Y	2	45.6
CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO/7/SOKOLL/WBLL1 PTSS16Y00074S-0B-099Y-099M-22Y-0Y	2	40.6
ALTAR 84/AE.SQUARROSA (237)//2*KUTZ PTSS16B00017T-099Y-099M-16Y-0B-0Y	2	40.1
SOKOLL/5/W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1/6/SOKOLL/3/PASTOR//HXL7573/2*BAU PTSS12SHB00020T-0TOPB-099Y-099B-6Y-020Y-0B	1	40.1
Trial:1R		
COLIBRI/ACACIA TR13CS148-100R-100C-0C-5C-0C-1R	3	42.2
COLIBRI/3/WBLL1/FRET2//PASTOR TR07CS225-12R-0C-0R-5C-0R-0C	3	42
ENE/ZITA/3/WBLL4/KASOS//PASTOR/8/TACUPETO F2001/6/CNDO/R143//ENTE/ TR11CS152-0R-0C-0R-0C-9R-0C-0R	1	40.9
HALITA/COLIBRI//THELIN/2*WBLL1 TR13CS226-100C-100C-0R-4C-0R-0C	1	40.8

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Monitoring of the Fall Armyworm (*Spodoptera frugiperda* Walker) Moth for the Determination of Efficient Chemical Control in *Zea mays* L.

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ABSTRACT

Objective: To determine the population dynamics of the fall armyworm (FAW), identify the critical period of highest infestation and to determine the number of agrochemicals applications for its control.

Design/methodology/approach: A monitoring and capture of male FAW moths was conducted using plastic traps with sexual attraction pheromones. A daily count of captured moths was performed during the crop season, the data were plotted to determine the period of highest infestation and the optimal timing for chemical control. Additionally, the number of insecticide applications for FAW control was evaluated, with treatments including none (T0), one (T1), two (T2), three (T3), and four (T4) applications. A randomized complete block design with nine repetitions was used. Before each application, the number of plants with visible damage and its intensity were counted using the Davis visual scale. Statistical analysis of the measured variables was conducted.

Results: The results showed that moths were evenly distributed across the planted surface, and two periods of higher infestation were identified: between 32 to 35 and 70 to 76 days after planting, respectively. The biological cycle of the FAW was between 38 to 41 days. The analysis of variance showed statistical differences ($p \leq 0.001$) among the treatments.

Findings/conclusions: Using plastic traps with sexual attraction pheromones is an efficient method for capturing, monitoring, reducing the population, estimate the length of the biological cycle, and identifying the highest infestation period of the FAW. Moreover, two insecticide applications during the periods of highest infestation resulted in optimal control of FAW.

Keywords: Sex pheromones, insecticides, trapping, *Zea mays* L.

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INTRODUCTION

In Mexico, maize is the most important crop due to its production value and its social and cultural significance. Currently, approximately 28 million tons are produced, which is insufficient to meet the internal demand of nearly 46 million tons. Consequently, 17 million tons were imported, making Mexico the second-largest maize importer in the world (SIAP, 2022). Therefore, it is urgent to develop research strategies to increase production and grain yield. One of the main causes of loss in grain yield and quality are pests and diseases; thus, rational management and control of these factors is essential to enhance production and profitability of the crop. The fall armyworm (*Spodoptera frugiperda*) is one of the most significant pests affecting maize in Mexico. This pest is constantly adapting and is distributed throughout the Americas. In recent years, it has spread to most parts of the world, causing substantial economic losses in Africa and Asia (FAO, 2020). Although this insect has a preference for grasses, it can infest over 186 crops (Casmuz *et al.*, 2010). Previously, in Mexico, it caused significant damage in tropical and subtropical regions of the country. Currently, significant infestations are found in transitional regions and high valleys, at altitudes ranging from 1900 to 2600 meters, demonstrating the insect's adaptive changes to tolerate these climatic conditions (Casmuz *et al.*, 2010). In addition to being considered a highly aggressive pest, with significant infestations at all phenological stages of the crop, it has adapted its feeding habits. Initially, its damage was concentrated only on the whorl of the plant; however, it can now act as a defoliator, borer, cutter, and during the reproductive stage, it causes damage to the ear and tassels. Furthermore, during the grain-filling stage, it feeds on the cob, causing damage to the maize, which results in economic losses for the producer. It is reported that during critical periods, production losses can range between 10% and 30% (Bahena, 2020). One alternative for its control is the use of insecticides; however, excessive and improper use leads to control inefficiency, insecticide resistance, increased environmental contamination, and higher production costs, which ultimately results in decreased crop profitability (Bahena & Velázquez, 2012). To mitigate these issues, new control methods have been developed, such as Integrated Pest Management (IPM) and Agroecological Pest Management (APM). These approaches integrate and combine monitoring techniques with agroecological control methods, including the use of biological or chemical insecticides with low environmental impact. One of these alternative tools for monitoring and controlling fall armyworm is the use of traps with sex pheromones, which exploit the attraction and confusion of male moths during their reproductive stage. The use of pheromones is considered an effective, low-impact environmental control technique, as it allows for the reduction of populations through the capture of moths and constant monitoring of larval periods in the field. This helps to identify critical infestation periods, determine the duration of the biological cycle, and make informed applications of chemical products for optimal control. The objective of this research was to monitor male fall armyworms to determine the population dynamics and identify the critical period of highest infestation and damage to the crop. Additionally, it aimed to determine the number of insecticide applications and the optimal timing for chemical control during the maize cycle.

MATERIALS AND METHODS

This research was conducted at the Centro Altos de Jalisco Experimental Field of the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), located in Tepatitlán, Jalisco, Mexico, at an altitude of 1930 meters within the Agroecological Transition Zone. The geographic coordinates are 20° 52' 23" North latitude and 102° 42' 45" West longitude. The experiment was established on June 3 and 4 during the spring-summer (SS) agricultural cycle of 2021 and consisted of two stages.

Stage I. Moth Capture and Monitoring

The maize experiment was established in a 11,760 m^2 plot with a population density of 78,000 plants ha^{-1}. The agronomic management applied followed the recommendations provided by INIFAP for the study region (Chuela *et al.*, 2011). The traps for capturing male moths were placed immediately after planting. These traps were made from square containers, with openings on three of the four side faces and a 15 cm deep bottom, acting as a basin, which was filled with water + biodegradable soap. The traps were secured to a wooden stake and positioned at a height of 1.20 meters above the ground. A wire was threaded through the top face of the container, where a capsule containing the sex attraction pheromone was placed (Figure 1). Each pheromone trap covered a circular area of 2,500 m^2, therefore, four traps were sufficient to cover the experimental plot. The soapy solution was changed every seven days, and the capsules were replaced every 28 days, as recommended by the manufacturer.

To understand the population dynamics of the adult fall armyworm, a daily count and record of the moths captured in each trap was conducted. The daily capture data were grouped by week and plotted to observe the frequency distribution and identify the infestation peaks, which were used to schedule insecticide application dates. Additionally, a mean comparison was conducted between the number of moths captured per trap to observe the dispersion of the moths within the planted area.

Stage II. Determination of the Number of Chemical Insecticide Applications for Fall Armyworm Control

To determine the number of applications required for the chemical control of the fall armyworm, an additional experiment was established in the same location during the same SS 2021 production cycle. Five treatments were evaluated as follows: T0=Control (no applications), T1=one application at 30 days after sowing (30 DAS), T2=two applications (30 and 37 DAS), T3=three applications (30, 37, and 51 DAS), T4=four applications (30, 37, 51, and 58 DAS). A randomized complete block design with nine replications was used (Table 1). The total size of the experimental unit was eight rows, each 16.5 meters long with 0.80 meters of spacing between rows. The two central rows were used as the useful plot to avoid edge effects. A white-grain maize hybrid was used for the treatment evaluations, which was sown mechanically at a density of 70,000 plants ha^{-1}. Agronomic management

Figure 1. Appearance of the traps and their placement in the plot, supported on a wooden base. Left: Placement of the capsule with the pheromone. Center: Placement of the trap in the field. Right: Surface of the solution with captured male moths.

Table 1. Number of moths captured per trap during the crop production cycle.

Month	Trap 1	Trap 2	Trap 3	Trap 4	Total
June	309	298	458	199	1264
July	511	400	477	183	1571
August	174	290	95	532	1091
September	60	54	25	78	217
October	0	0	0	0	0
November	0	0	0	0	0
December	0	0	0	0	0
Total	1054	1042	1055	992	4143
Daily average	8.7	8.6	8.7	8.2	8.6

was carried out according to INIFAP's recommendations for maize cultivation in the study region (Chuela *et al.*, 2011), except for the number of insecticide applications for the chemical control of the fall armyworm.

In each treatment, the total number of plants was counted, and 10 plants were randomly selected. The active ingredients of the insecticide were Emamectin Benzoate+Lambda-cyhalothrin, applied at doses of 150 mL + 375 mL ha^{-1}. Applications were made using a 15 L manual backpack sprayer equipped with Lurmark 30 FCX04® full cone nozzles. The product dosage for each treatment was calibrated by measuring the output per backpack, based on a rate of 220 liters of water per hectare, which corresponded to 10.5 mL + 21 mL of Emamectin Benzoate + Lambda-cyhalothrin, respectively.

Study Variables

For the 10 plants selected within each treatment and replication, the following data were collected: Number of Plants with Visible Damage: The number of plants with visible damage: This was counted for each treatment, using a scale of 0 and 1, where 0 indicates no damage and 1 indicates damage present. Damage Intensity: For plants with visible damage, the damage was assessed using the visual scale from 1 to 9 (Davis *et al.*, 1992). In this scale, values from 1 to 3 represent minimal damage caused by larvae less than 1.0 cm in length. Values from 4 to 6 represent significant damage caused by larvae from 1.1 to 2.0 cm in length, which are already established in the plant's whorl. Finally, values from 7 to 9 represent severe damage to the plant caused by larvae greater than 2.1 cm in length (Figure 2).

Statistical Analysis

The data on the presence, absence, and intensity of damage caused by the fall armyworm were analyzed using an average filtering process of the number of plants with damage at any period of the crop for each treatment. This generated a new database with the number of damaged plants for each treatment. This new database was subjected to an analysis of variance (ANOVA) to determine the optimal number of applications for the control of the fall armyworm. Additionally, a post-hoc HSD-Tukey test was performed to determine

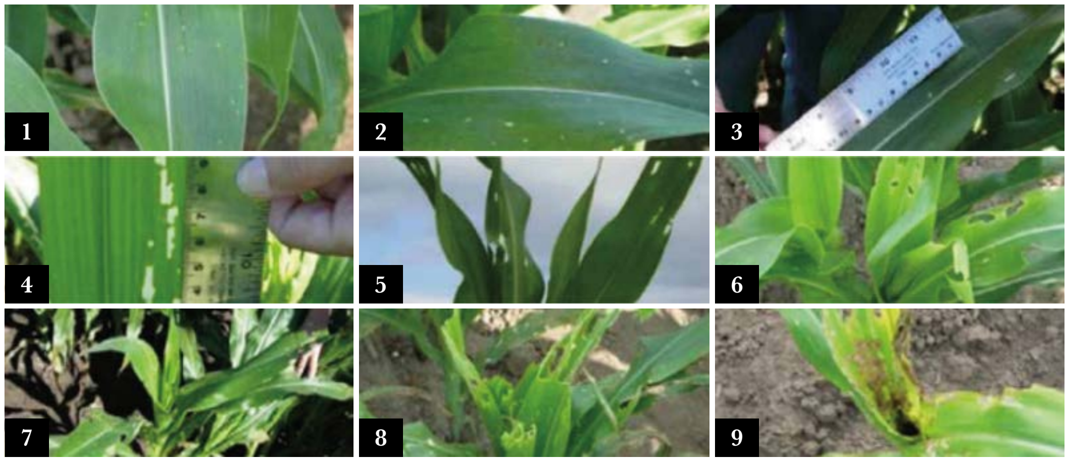

Figure 2. Davis *et al.* (1992) scale for the visual assessment of damage caused by the fall armyworm.

the differences between treatment means. Statistical analyses and graphs were conducted using R and RStudio software (R Core Team, 2022).

RESULTS AND DISCUSSION

Capture and Monitoring of Moths

A total of 4,143 moths were captured during the maize production cycle (June to December). July was the month with the highest capture of moths (1,571), while no moths were captured from October to December. Considering only the months from June to September (when moths were captured), the average daily capture per trap was very similar and showed no significant differences (Table 2). Similarly, considering only the months of moth capture, the weekly average was 259 moths captured. This capture result was lower compared to the average of 519 moths per week reported by Salazar *et*

Table 2. Comparison (Tukey≤0.05) of the treatment means for the number of insecticide applications based on the number of plants with damage.

Number of applications	Difference	Minimum	Maximum	Adjusted P-value	
T0 *vs.* T1	−9.1	−15.5	−2.7	0.002	*
T0 *vs.* T2	−14.2	−20.6	−7.8	1.5×10^{-6}	**
T0 *vs.* T3	−19.1	−25.5	−12.7	0.001	**
T0 *vs.* T4	−19.5	−26.0	−13.2	0.001	**
T1 *vs.* T2	−5.1	−11.5	1.3	0.172	ns
T1 *vs.* T3	−10.0	−16.4	−3.6	5.8×10^{-4}	**
T1 *vs.* T4	−10.4	−16.8	−4.0	3.2×10^{-4}	**
T2 *vs.* T3	−4.8	−11.3	1.5	0.208	ns
T2 *vs.* T4	−5.3	−11.7	1.1	0.142	ns
T3 *vs.* T4	−0.4	−6.8	6.0	0.999	ns

*=Significant at the 5% probability level; **=Significant at the 1% probability level; ns=Not significant.

al. (2020). These observed differences are primarily due to the study region. In the case of Salazar *et al.* (2020), the study was conducted in a tropical region (70 m altitude), where high populations of the fall armyworm are recorded compared to regions at altitudes close to 2000 m. During the vegetative and early reproductive stages of the plants (June to September), the highest number of moths was captured per month (Table 2). Similar results were observed by De la Cruz *et al.* (2018) and Salazar *et al.* (2020), who recorded higher moth populations and greater damage to the crop during the vegetative stage. On the other hand, in September (flowering stage), there was a drastic decrease in the number of moths captured, with a total of 217 moths from the four traps. However, this capture was significant because it initiates new populations of fall armyworm larvae that affect the reproductive structures of the plants (ears and silk). Nevertheless, these populations do not become very high because the larvae have a lower probability of survival due to a lack of food, the presence of other entomophagous insects, and the unfavorable environmental conditions that create non-optimal environments for the insect to reproduce, forcing it into a state of dormancy (Hardke *et al.*, 2015). In this sense, September was considered the final capture stage for moths; however, traps continued to be monitored for the remaining three months. Analyzing the total number of moths captured during the growing season (4,143), it is important to highlight two things: 1) the efficiency of the traps in reducing larval populations and 2) the identification of the period of highest adult incidence.

In the first case, if all 4,143 captured male moths had mated, they would have resulted in 4,143 ovipositions (100 eggs). If at least one larva from each oviposition reached adulthood, this would have prevented approximately 4,143 individuals from causing damage daily across the field, potentially resulting in an infestation exceeding 50% of the crop.

Based on the daily record of the number of moths captured, it was estimated that in the study area, the biological cycle of the insect lasts from 38 to 41 days (Figure 3). These results align with Bahena (2020), who reported that the biological cycle of the fall armyworm ranges from 28 to 60 days; however, it is noted that this duration depends on the agricultural zone and the crop production cycle. On the other hand, Salazar *et al.* (2020) report that in tropical areas, the biological cycle of the fall armyworm is 30 days per generation. In this context, two generations were identified during the months of June to September, which is consistent with the findings of Rojas *et al.* (2004) and Salas-Araiza *et al.* (2018), who reported that the fall armyworm exhibited two generations during the maize production cycle. They also mention that the highest infestation frequencies found serve as a reference for chemical control. However, it is essential to know the larval instars of the fall armyworm, the duration of its biological cycle, and the recommended doses and application guidelines for insecticides to achieve optimal control. The first peak of infestation occurred at 35 DAS, with 1,400 moths captured over the course of one week (Figure 3). The second peak of infestation occurred at 75 DAS, representing the second generation of moths, during which 896 moths were captured over the course of one week (Figure 3). At this peak, the moth capture was 36% lower than in the first peak. This reduction could be attributed to the timely chemical control implemented during the first generation of moths. Based on the dynamic data, the phenological stages of the crop with the highest frequency of moths and the greatest damage were identified. It was observed

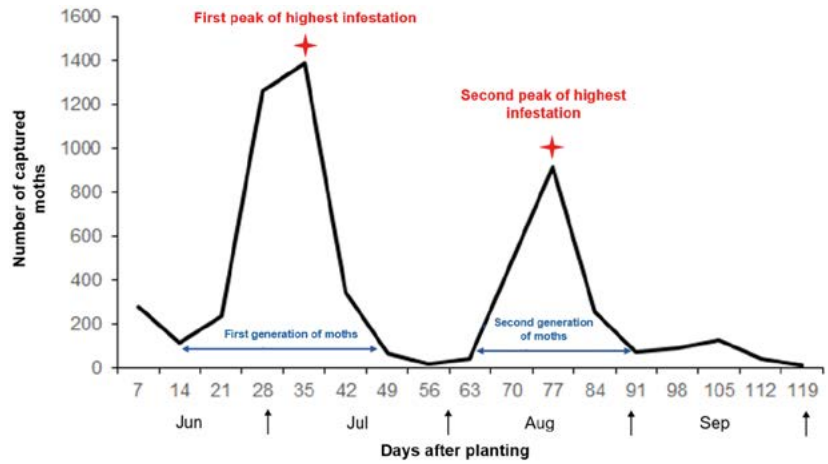

Figure 3. Weekly distribution of fall armyworm moths captured in pheromone traps during the months of peak insect presence (June-September).

that the highest frequency of infestation occurred during the vegetative stage, between the V3 and V4 stages of the crop. Therefore, the first larvae would be expected around 26 DAS. However, considering that the most effective chemical control is achieved during the first three larval instars, these would appear around 35 DAS. Thus, it is recommended to apply the insecticide between 30 and 35 DAS to control both adults and the early larval instars emerging from the hatched eggs. Furthermore, it is expected that the insecticide applied would cover a period of 21 days, which would help reduce the larval population. The second peak of infestation occurred between the V14 and VT phenological stages of the crop. During this infestation, the larvae cause damage to the foliage, flag leaf, ear, and cob. In this regard, Salas-Araiza *et al.* (2018) found a second peak of infestation of fall armyworm larvae during the same phenological stages. The duration of the second peak of infestation was 21 days; therefore, the second application is recommended between 70 and 75 DAS to control the early larval instars and prevent damage to the reproductive structures of the crop. However, due to the phenological stage of the crop, plant height, and the toxicity of the insecticides, manual application of chemical products is limited. Consequently, consideration should be given to using drones or machinery adapted for this purpose. On the other hand, the adaptability of the fall armyworm and its diverse feeding habits were confirmed, as it was observed cutting and boring into plants during the seedling stage, defoliating during the vegetative stage, and feeding on the ear, husks, and cob during the reproductive stage. Salazar *et al.* (2020) confirm this broad adaptability of the fall armyworm and emphasize the importance of implementing effective control measures to minimize crop damage.

This research confirmed that capturing and constantly monitoring fall armyworm moths using pheromone traps is a useful tool for determining population dynamics, estimating the duration of the biological cycle, defining critical stages of highest crop damage, and pinpointing the optimal time for chemical control of the fall armyworm. Similarly, Malo and Rojas (2020) highlighted the efficiency of using pheromone traps as an effective method for controlling fall armyworm in maize.

Determination of the Number of Chemical Applications for Fall Armyworm Control

The analysis of variance only showed statistical differences ($p \leq 0.001$) between treatments, indicating uniformity among repetitions. The number of plants with visible fall armyworm damage varied according to the number of applications made. The control (T0) had the highest percentage of damage, 84.2%, compared to one (T1), two (T2), three (T3), and four (T4) applications, which had percentages of 66%, 55.8%, 46%, and 45.1%, respectively (Figure 4). This indicates that the number of chemical insecticide applications reduced the number of plants with visible fall armyworm damage, but the differences in damaged plants between T2, T3, and T4 do not justify performing a third or fourth application.

There were significant differences ($p \leq 0.05$) in the comparison of means between T0 and the insecticide treatments. Therefore, applying chemical products has a significant effect on the control of the fall armyworm. Treatment T1 did not show differences compared to T2; however, T1 did have significant differences ($p \leq 0.05$) compared to T3 and T4, indicating better control when more than one application is made. On the other hand, T2 showed differences ($p < 0.001$) compared to T0, but did not show differences ($p \leq 0.05$) compared to T1, T3, and T4 (Table 4). This means that performing two applications statistically provides the same control of the fall armyworm as performing three or four applications. Therefore, based on these results, it is most advisable to perform two applications, thus avoiding excessive use of chemical products and labor. These results agree with those found by Vélez *et al.* (2021), who used the economic threshold (points of highest infestation) as their treatment guide, which provided the best control. The same authors indicate that using the economic threshold helps determine the timing for starting control strategies to prevent significant damage and reduce the number of chemical applications.

According to Mora and Paulo (2019), a higher number of chemical applications at 12-day intervals resulted in better control. However, there were no significant differences when the interval between applications was increased to every 30 days, nor did they find significant differences in yield. On the other hand, the benefit/cost ratio is higher when the

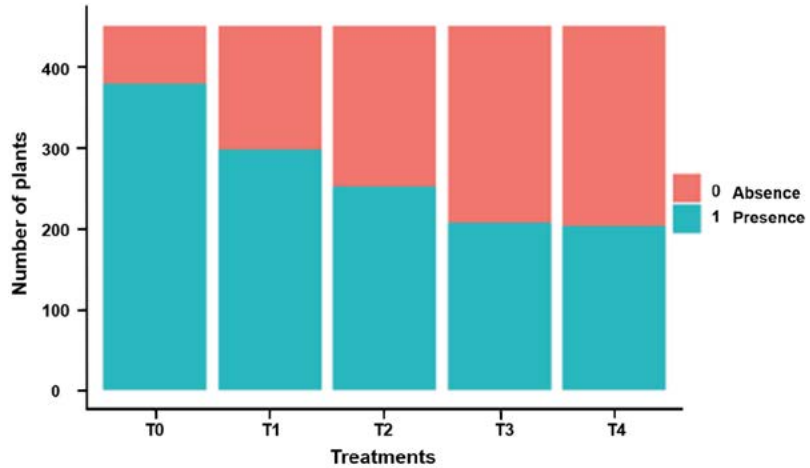

Figure 4. Presence and absence of visible damage with respect to the number of chemical applications.

number of applications is reduced, and it also represents less pollution and environmental impact (Mora and Paulo 2019). In (Figure 5), the decrease in the number of damaged plants with the increase in the number of chemical applications is observed. The number of plants with visible damage within each repetition varied according to the number of applications performed. In T0, it was observed that, on average, the number of damaged plants ranged from 40 to 45 per repetition, while T4 showed only 5 to 35 damaged plants, with the lowest average of 22 plants per repetition. These results are similar to those found by Mora and Paulo (2019), where the percentage of damage statistically decreased as the number of applications increased.

Damage Intensity

Regarding the intensity of the damage assessed using the Davis *et al.* (1992) scale, a decrease in damage intensity was observed as the number of applications increased. The control (T0) exhibited the highest percentage of damaged plants (84.2%) and an average damage intensity of 2.9. This was followed by treatment T1, which had 66% of damaged plants and an average damage intensity of 2.4. Treatment T2, with 55.8% of damaged plants, showed an average damage intensity of 2.1. Finally, treatments T3 and T4, with 46% and 45.1% of damaged plants respectively, had the lowest damage intensity values at 2.0 and 1.9 on the Davis scale (Table 3).

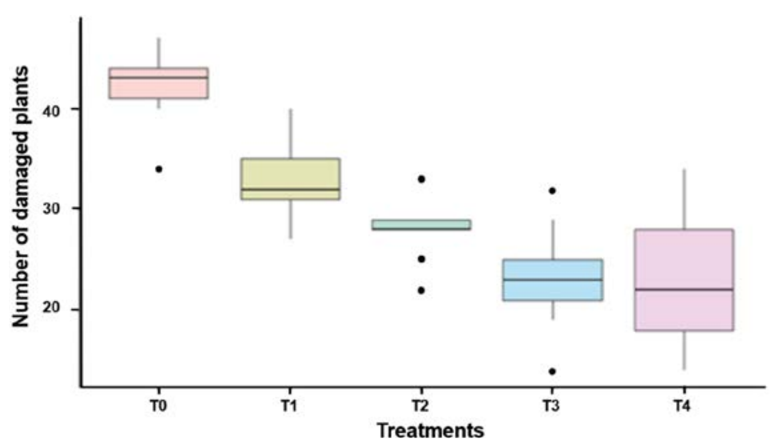

Figure 5. Average number of damaged plants per repetition depending on the number of applications performed.

Table 3. Number of Plants with Visible Damage and Damage Intensity (Davis Scale) with Respect to the Number of Insecticide Applications.

Treatment	Plants evaluated	Plants with visible damage (%)	Damage intensity (Average)*
T0	450	84.2	2.9
T1	450	66.0	2.4
T2	450	55.8	2.1
T3	450	46.0	2.0
T4	450	45.1	1.9

* The average damage intensity evaluated with the visual scale of Davis *et al.* (1992).

Additionally, it was observed that in T0 and T1, there were damage intensities ranging from 7 to 9 with 3.4% and 1.7% of plants showing visible damage, respectively, while T2, T3, and T4 did not exhibit plants with this level of damage intensity. Similarly, for damage intensities of 4 to 6, T0 and T1 had the highest damage percentages with 22.7% and 14.5%, respectively, while T2, T3, and T4 had damage percentages of 5.2%, 7.7%, and 4.4%, respectively (Table 4). This indicates that the severity of damage increases with fewer insecticide applications. Finally, for damage intensities of 1 to 3 on Davis's scale, T0 and T1 showed percentages of 7.9% and 83.8%, respectively, while T2, T3, and T4 had percentages of 94.8%, 92.3%, and 95.6%. Despite the high percentages of damage in the range of 1 to 3, this indicates that with a higher number of insecticide applications, the severity of damage decreases (Table 4).

Table 4. Damage intensity on the Davis scale depending on the number of chemical control applications.

Treatment	Damage intensity (Davis scale (%))			
	1 a 3	4 a 6	7 a 9	Total
T0	73.9	22.7	3.4	100
T1	83.8	14.5	1.7	100
T2	94.8	5.2	0	100
T3	92.3	7.7	0	100
T4	95.6	4.4	0	100

Considering that the maximum value on Davis *et al.* (1992) scale is 9, the control treatment (T0) had the highest damage intensity (8), confirming that not implementing chemical control for the corn borer can result in greater losses in yield and grain and/or forage quality. In this regard, Mora and Paulo (2019) found that when no insecticide applications were made, the average damage on the Davis scale was 6.5, and similarly, increasing the number of insecticide applications reduced the damage.

CONCLUSIONS

The use of pheromone traps is an effective tool for capturing, monitoring, reducing the population, estimating the biological cycle duration, and identifying peak infestations of the adult male fall armyworm. The population dynamics help determine the optimal time for chemical control. Increasing the number of chemical applications reduced the number of damaged plants and the severity of damage caused by the fall armyworm. Applying insecticide twice during the peak infestation periods is sufficient to achieve optimal control of the fall armyworm.

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Colegio de Postgraduados

Challenges and Opportunities in the Specialization of Maize Cultivation

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ABSTRACT

Objective: To identify current scientific information on maize (*Zea mays* L.) in relation to the challenges and opportunities associated with the specialization of this crop.

Design/methodology/approach: A bibliographic search was conducted in high-impact journals focusing on the difficulties and opportunities in maize cultivation.

Results: Several key challenges in maize cultivation were identified, including the impact of climate change, limited access to technologies for small and medium producers, variability in grain price, and the availability of improved seed to increase yields in their reproductive zones. One potential solution involves access to improved short-cycle varieties with tolerance to adverse factors (both biotic and abiotic), combined with sustainable agricultural techniques such as conservation agriculture.

Limitations on study/implications: Despite existing research on maize cultivation, its composition, nutritional contribution and economic importance, the profitability of this crop is affected by factors such as price variation, climate change, and the incidence of pests and diseases. Therefore, further research is needed to identify varieties with more competitive markets to enhance sustainability.

Findings/conclusions: Maize is a globally important crop with industrial applications and uses for both human and livestock consumption. The increasing demand underscores the need to improve its performance and profitability. A strategy to increase production involves promoting diversification through the specialization of varieties with more competitive markets and the adoption of sustainable agricultural techniques.

Keywords: Maize, stress, climate change, hybrids, profitability.

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INTRODUCTION

Globally, maize (*Zea mays* L.) is recognized as one of the most important cereals, playing a fundamental role in human consumption and the livestock sector. Projections for the 2030 decade estimate that maize will be the crop with the highest production, consumption, and commercialization worldwide (Erenstein *et al.*, 2022).

The prominent position of maize cultivation is directly linked to its versatility in uses and adaptability. In addition to its significant role in nutrition, maize also represents a key raw material in the industry, as it is used in edible products, biofuels, plastics, and other

industrial products (Ruan *et al.*, 2019). In countries like Mexico, maize is not only a staple food (Eakin *et al.*, 2014) but also a fundamental part of the country's history and culture (Cervantes *et al.*, 2014). Mexico hosts a great diversity of maize, with 59 recognized races (Duncan *et al.*, 2019) and thousands of varieties adapted to different climatic conditions and altitudes, representing approximately 50% of the genetic diversity of maize in the American continent (Ureta *et al.*, 2020).

The increase in global population and the growing demand from industries such as food and energy pose a significant challenge to increase the productivity and efficiency of maize cultivation (García-Lara and Serna-Saldivar, 2019; Ruan *et al.*, 2019). To meet this demand, it is necessary to innovate in crop management to explore specific market niches that encourage its development. One of the promising strategies focuses on the specialization of cultivation, which involves the development of maize varieties adapted to specific needs in terms of yield, nutritional quality, resistance to pests, diseases, and adverse environmental conditions (Bekele and Rao, 2013), coupled with special technological applications of industrial interest.

While the specialization of maize cultivation has generated significant advances such as the development of hybrids and improved varieties to meet the specific demands of the industrial sector, it also poses important challenges regarding the loss of genetic diversity (Darrah *et al.*, 2019). Therefore, it is essential to adopt a balanced approach that allows for the preservation of this diversity while also offering the market the advantages and opportunities of maize cultivation specialization. In this context, the aim of this analysis was to identify the current scientific information on maize (*Zea mays* L.) regarding the challenges and opportunities associated with the specialization of this crop.

MATERIALS AND METHODS

A search on the specialization of maize (*Zea mays* L.) cultivation was conducted using the keywords: *Zea mays* L., maize cultivation, maize cultivation specialization, maize issues, climate change, food security, genetic improvement, and maize uses, in the databases of ScienceDirect, Wiley, Springer, Google Scholar, Scielo, and MDPI. Scientific articles from 2013 to 2024 were selected, prioritizing updated information on the topic.

RESULTS AND DISCUSSION

Challenges in maize cultivation; climate change in production

Constant changes in markets exert significant pressure on the agricultural production sector. In particular, the increase in demand and uncertain prices of maize grain drive the search for alternatives, such as cultivation specialization. This strategy aims to obtain economic advantages, improve production efficiency, and access higher value-added markets. However, this process faces a series of challenges that require attention and appropriate solutions (Bekele and Rao, 2013).

One of the greatest challenges for agriculture is climate change, which is expected to have increasingly intense effects on cultivation environments. In the first two decades of the 21st century, a global surface temperature increases of 0.99 °C compared to the 1850-1900 period has been recorded, along with an increase in CO_2, CH_4, and N_2O levels due to intense human activity (Intergovernmental Panel on Climate Change: IPCC, 2023). This has increased the incidence of climatic events such as droughts, variations in precipitation (Sharma *et al.*, 2022), floods, tropical cyclones (Murray-Tortarolo *et al.*, 2018), heatwaves, and cold waves (IPCC, 2023). Together, these factors increase the incidence of damage from biotic factors in crops like maize (Erenstein *et al.*, 2022), and pose future displacements of pests and diseases, along with a reduction of beneficial soil microorganisms (Sharma *et al.*, 2022).

In Mexico, a reduction in rainfall distribution and quantity, along with an increase in temperature, more droughts, and tropical cyclones is forecasted (Cervantes *et al.*, 2014). These phenomena will affect maize productivity, projecting a near 10% reduction in Latin America by 2055 (Murray-Tortarolo *et al.*, 2018).

Specifically, increased temperature has been associated with a decrease in maize yield (Ureta *et al.*, 2020), as it directly affects the vegetative period, pollination, and grain filling (Noriega-Navarrete *et al.*, 2021). This represents an inherent risk and directly impacts food security. Food security aims to ensure that, for foods such as maize, there is supply, access, availability, and affordability exist to achieve consumption and nutrition for the population. Given the high dependency on this crop in sectors such as livestock and industry, production for human consumption is likely to be reduced.

Additionally, it is relevant to note that climate change has a significant effect on rain-fed agriculture. In Mexico, approximately 80% of the maize cultivation area is dependent on these conditions (Cervantes *et al.*, 2014). Authors such as Ureta *et al.* (2020) mention that local varieties face greater difficulties adapting to higher temperatures and combined with reduced water access during flowering and grain filling stages, this results in a negative impact on yield (Sharma *et al.*, 2022).

Maize Production by Small and Medium Farmers; Grain Price Variation and Imports

Another aspect to consider in the challenges is that at least one-third of maize production in Mexico comes from small producers, whose cultivation areas are less than 5 hectares (Ureta *et al.*, 2020). This production is mainly focused on self-consumption, mostly under a rain-fed production system (Ibarrola-Rivas *et al.*, 2020). Producers face significant limitations in available resources such as access to inputs, improved seeds, agrochemicals, and fertilizers. They also have limited agricultural mechanization capacity and access to competitive markets (Eakin *et al.*, 2014). In contrast, in countries such as the United States, China, and Brazil, large producers have access to technology, extensive land, and hybrid and genetically modified seeds, which gives them a considerable advantage in crop efficiency and profitability (Erenstein *et al.*, 2022).

In the international market, Mexico's dependence on maize creates constant concern for small and medium producers due to falling prices. For example, at the beginning of 2024, a large supply of maize from other producing countries reduced the price from $244.08 to $182.47 per ton, representing a decline of more than 20% (García, 2024). These variations are related to market expectations about increased demand, speculation from major traders, and global maize supply. In a speculative environment, production cannot be estimated accurately, which disrupts supply and impacts maize marketing prices (Shobande and Shodipe, 2021), placing national production at a significant disadvantage.

Low maize prices leave producers with minimal or no profit margins, affecting food security in maize-dependent countries like Mexico and impacting other industries related to this cereal (Sayed and Auret, 2019). This volatility in maize prices is closely linked to various factors such as climate change, tariff and import costs, production costs, and productivity, among others. In relation to the major maize producers, the United States, Brazil, and Argentina account for approximately 70% of global maize exports (Ruan *et al.*, 2019; Sayed and Auret, 2019).

Although Mexico is the seventh-largest producer worldwide (SADER, 2023), it shows continuous dependency. In 2022, maize imports reached nearly 17 million tons, equivalent to 57.1% of national production (Ramírez-Díaz *et al.*, 2023). These imports are primarily of yellow grain destined for the livestock sector.

The Challenge of Genetic Improvement and Transgenic Materials

Regarding the development of improved maize germplasm, this has marked a turning point in the agro-food production system of this crop. The development of maize hybrids involves the selective combination of inbred parent lines to find heterosis or hybrid vigor (Tripathy *et al.*, 2017). This process requires years of work by breeders in the search for parents that contribute the necessary characteristics to increase yield (Fromme *et al.*, 2019), tolerance to biotic and abiotic factors, higher plant density (Haegele *et al.*, 2013), as well as adaptability and ease of production (Kutka, 2011). This involves a considerable investment in resources, time, and research.

Additionally, water supply, soil type, and nutrient availability must be considered (Assefa *et al.*, 2016). These conditions are not present in all environments, so varieties must be carefully selected for specific regions, representing a constant challenge for breeders. It should also be considered that intensive production systems can contribute to soil degradation in the medium and long term due to the reduction of organic matter and nutrients, affecting soil fertility. This problem increases the dependence on inputs such as fertilizers, which, when used for extended periods, will have negative impacts on the environment (Erenstein *et al.*, 2022).

To increase yields and crop resistance, transgenic maize varieties have also been developed. These genetically modified varieties contain genes that confer resistance to insects, tolerance to herbicides such as glyphosate, and some tolerance to abiotic factors such as drought. These advances are mainly adopted in major producing countries such as the United States and Brazil, where transgenic maize cultivation represents about 90% (Duncan *et al.*, 2019). This approach focuses primarily on benefits such as increased yields, reduced use of agricultural inputs (pesticides), and resistance to pests and diseases (Yadava *et al.*, 2017). However, the adoption of these materials can have an ecological, socioeconomic, and cultural impact in a country like Mexico, which is the main center of origin and domestication of maize, as its use could cause contamination of native maize with transgenes (Luna and Altamirano, 2015). In this regard, the acceptance and regulation of these crops in Mexico generates debates about the effects and repercussions on food security, biodiversity, and farmers' sovereignty (Erenstein *et al.*, 2022), suggesting that the acceptance of genetically modified material may not be viable or imminent for future scenarios. Currently, approximately two-thirds of the production comes from open-pollinated local varieties, valuable for their genetic diversity, adaptation, and particular characteristics (Duncan *et al.*, 2019).

In the maize improvement approach, Quality Protein Maize (QPM) varieties have been developed, with the aim of enriching diets for human consumption by increasing the content of essential amino acids (lysine and tryptophan). However, the initial experiments exhibited undesirable characteristics such as unpleasant taste, soft and floury endosperm, leading to increased susceptibility to pests and diseases, and reduced yield at harvest (Tripathy *et al.*, 2017). This case highlighted some of the difficulties encountered in obtaining specific desired characteristics in the crop. Currently, efforts continue to develop protein-quality maize with agronomic characteristics and acceptance for commercialization.

In this context, the research and adoption of viable technologies are fundamental for the sustainable production of maize. To adequately address the identified challenges, an integrated approach is required, which also includes sustainable agricultural practices (conservation agriculture), as activities such as plowing and harrowing have caused the deterioration of soil structure and quality (Martínez-Gamiño *et al.*, 2020). Additionally, there is a need to provide access to technologies for small producers, training programs, and agricultural sector policies that promote inclusion and equity in access to more competitive markets for maize. These measures can significantly contribute to improving and incentivizing maize production.

Opportunities in Maize Cultivation

The global importance of maize cultivation is based on its versatility and broad usage, attributed to its genetic diversity. Its applications range from livestock feed to food and non-food uses. This crop plays a crucial role in food security and nutrition, contributing directly and indirectly to the human diet (Ranum *et al.*, 2014). For example, it is estimated that approximately 3 kg of maize and soybean grain is estimated in ruminant diets that produce around 1 kg of meat (Erenstein *et al.*, 2022), mainly due to the energy contribution of lipids and proteins present in the grain.

To strengthen sustained maize production and contribute to future food security, it is essential to involve and integrate research, development, and technology transfer, as well as the implementation of public policies and training for producers to identify development opportunities for maize cultivation. In this regard, it is important to clarify that information on maize for special uses is limited. According to the SIAP database (2023), the existing information on maize production is categorized into three major groups: green fodder maize, grain maize, and popcorn maize. For this last case, the data on the platform reported a production of 246.72 tons of popcorn maize. Regarding grain maize, 27,549,917.53 tons were recorded, of which, as specialty maize, only the categories of colored maize (59,747.74 tons), blue maize (12,073.39 tons), and pozole maize (26,960.81 tons) are presented. These data allow us to see that the reported production of specialty maize in Mexico represents less than 0.5% of the national production, with these types of maize having the highest commercialization value. With this in mind, the promotion of specialty maize is proposed as an opportunity to boost production and the economic advantages that they can bring. To achieve this, specific programs must be developed and collaboration between academic institutions, governmental bodies, and the private sector to successfully drive this initiative.

Grain prices and crop specialization

Faced with eventualities, especially variations in the sale price of maize grain, various alternatives can be considered. Establishing a minimum sale price (Shobande and Shodipe, 2021), paying for risk coverage, or using sales contracts (Harčariková, 2018) are strategies that can be employed to minimize the effect of falling maize crop prices. Reyes-Santiago *et al.* (2022) mention that in Mexico, guaranteed prices are defined as an economic policy instrument aimed at increasing the price received by producers (above the market equilibrium) while maintaining the price for consumers (below the market level), with the government covering the difference. These same authors emphasize that guaranteed prices in Mexico encourage maize production, although in a conservative manner. They recommend interaction with other support programs for the agricultural sector, such as fertilizer subsidies. Regarding the price of maize grain, it should be noted that it varies according to the type of grain being sold. In this sense, those focused on specialized applications command better prices than those intended for the livestock sector. For example, in Mexico, in 2023, popcorn maize had an average rural price of $8,283.94 per ton, pozole maize $7,244.92 per ton, blue grain maize $6,406.64 per ton, while white and yellow grain sold for $6,253.34 per ton (SIAP, 2023). This highlights the opportunity presented by specialty maize as a proposal to diversify production and identify the most profitable markets for producers.

Under this approach, it is important to highlight the existing diversity in maize in terms of color, ranging from white to dark blue, and varieties of maize with unique characteristics such as sweet maize, waxy maize, high oil content, high amylose content, high protein content, among others (Serna-Saldivar, 2019). The use of these specialized crops offers advantages for more profitable trades and the promotion of niche markets, such as snacks, cereals, and preserves, among others.

Examples of Recognized Specialty Maize Types

The so-called specialty maize varieties are those that present specific characteristics making them useful for applications in both food and industrial sectors. These differences are closely related to variations in the endosperm, pericarp, and germ, as well as the starch composition and proximate analysis (Serna-Saldivar, 2019). These specific attributes provide the opportunity to identify and penetrate markets with higher profitability for maize.

Among these types of specialty maize of interest is the variety used for higher oil extraction. This component is primarily found in the germ of the grain. For this purpose, the oil content must be above 6% (García-Lara and Serna-Saldivar, 2019). This variety of maize has a high content of monounsaturated fats with a high content of oleic acid, which has demonstrated cardiovascular health benefits (Darrah *et al.*, 2019). Among the industrial advantages of fatty acids obtained from maize, oleic acid, for example, exhibits greater oxidative stability, thus a longer shelf life, making it suitable for frying processes and high-temperature conditions (Barrera-Arellano *et al.*, 2019).

In Mexico, a highly recognized type of specialty maize is the one used for pozole, which has specific characteristics such as large, starchy kernels, like Cacahuacintle, Ancho, and Western maize varieties. These kernels, after cooking, open like a flower and produce foam when boiled (pozolli comes from the Nahuatl word meaning "foamy"). This type of maize can be sold in its unprocessed form, which means selling the maize grain as-is without added value, as well as precooked and dehulled maize for pozole (García-Lara and Serna-Saldivar, 2019).

Also known as "popcorn maize," this is a yellow or white grain of maize with a high proportion of vitreous endosperm and a hard pericarp. When this type of grain is subjected to elevated temperatures, the moisture content turns into steam, causing the grains to explode. They exhibit a high endosperm expansion rate, expanding 30 to 40 times their original size (García-Lara and Serna-Saldivar, 2019). This type of maize is highly valued for producing snacks such as those consumed in cinemas and recreational events.

Another type of maize used for specific industrial purposes is known as "hard corn". It features a corneous or vitreous endosperm proportion greater than 90% and a high starch content (Tagmano *et al.*, 2016). This type of maize is utilized in the production of semolina, flours, and starch, and is employed in the production of ethanol and sweeteners such as maize syrup. These derivative products are obtained from the processing of maize grain, where the components are separated, and starch-forming endosperm serves as an essential substrate for glucose products (sweeteners) and those processed by fermentation (ethanol) (Ruan *et al.*, 2019).

Pigmented maize varieties are another example of a specialization in maize cultivation. These varieties exhibit colors such as red, blue, and purple in various shades and intensities. The presence of these pigments is found in the aleurone layer and the pericarp. The phytochemical compounds present in higher proportions are anthocyanins, which have demonstrated nutraceutical properties (García-Lara and Serna-Saldivar, 2019). In this regard, their application as pigments can be varied, both in the food and non-food sectors. The advantage is that, in addition to providing color, they also possess antioxidant activity. These examples of maize types provide a broad and complex landscape in which production can be promoted to enter these developing markets in Mexico or for export to countries where these maize types are already industrialized (biofuels, breakfast cereals, snacks, beverages, etc.). As mentioned, these markets require specific characteristics in maize, which are dictated by the industries that demand them. In this regard, current tools enable the improvement and development of materials with the highest possible quality.

Genetic improvement as a tool for specialization

The application of maize in processed products varies according to region, country, and local customs. In some countries, maize is a central part of the diet and is used in various edible products (Ranum *et al.*, 2014). However, maize specifically provides minimal amounts of essential amino acids such as lysine and tryptophan, representing a nutritional challenge. To address this problem, quality protein maize (QPM) varieties have been developed, which contain a higher proportion of these essential amino acids (Tripathy *et al*., 2017), with the aim of improving nutritional intake (Maqbool *et al*., 2021). The development of important varieties such as QPM represents a significant advance in genetic improvement and another example of maize crop specialization, which aims to address the nutritional deficiencies of populations that rely on this crop as their main food source (Tandzi *et al*., 2017). This approach highlights the opportunities that can contribute to boosting the agricultural sector and counteracting the nutritional deficiencies of the population.

Waxy maize, high-amylose, and sweet maize varieties are other examples of genetic modifications and improvements developed to provide specific characteristics to this cereal. In the food sector, waxy maize is a variety modified so that the main component of endosperm, called starch, is almost entirely amylopectin, with a content greater than 95% (in contrast to normal starch, which has approximately 75% amylopectin and 25% amylose). The characteristics of this type of starch, which are of industrial interest, are that when extracted through the wet milling process (in waxy maize), it allows for the formation of viscous pastes, low tendency to retrogradation, ease of enzymatic hydrolysis, and the formation of soft, transparent gels that easily disintegrate. Therefore, it is ideal for food applications such as desserts, frozen products, and dressings (García-Lara and Serna-Saldivar, 2019).

High-amylose maize (amylomaize) features a recessive gene that expresses high production of linear amylose, which is stored as starch in the endosperm. Its content ranges between 37% and 65%. Its industrial importance lies in the technological and functional properties provided by amylose, including the formation of rigid, opaque gels with a high tendency to retrogradation. Its potential applications are found in the textile, paper, adhesives, and biodegradable foam packaging industries, as well as in the confectionery industry (García-Lara and Serna-Saldivar, 2019).

Some varieties, such as sweet maize, are sought for consumption in their immature state. This variety has been developed through genetic modification and features recessive genes that reduce starch synthesis in the endosperm, resulting in maize kernels with a higher sugar content (Dong *et al*., 2019). This variety is consumed as maize on the cob, when the kernels are tender and have a high water and sugar content, providing the characteristic flavor (Singh *et al*., 2014). It is primarily consumed fresh but can also be found frozen, canned, and pickled, and is used in dishes, salads, sauces, etc. (García-Lara and Serna-Saldivar, 2019).

It should be recognized that, in addition to the characteristics provided by genetic improvement in maize, it has also contributed to increased and stable yields, particularly in areas prone to variations in water availability (Cooper *et al*., 2014), such as rainfed maize cultivation areas. In this sense, hybrid varieties show superiority in aspects such as efficient nitrogen use, higher leaf photosynthesis rates, increased plant density, and tolerance to adverse environmental conditions, all as a result of the rigorous selection process (Fromme *et al*., 2019). However, to ensure the optimization of available resources, they must be accompanied by appropriate agronomic practices that develop their potential, thus contributing to food security and the sustainability of agricultural production (Darrah *et al.*, 2019).

Industrial Uses of Specialty Maize

The primary use of maize is focused on livestock feed production, where it can constitute up to 70% of the diet (García-Lara and Serna-Saldivar, 2019). This cereal, in its dry form, has an approximate composition of proteins (≈9%), oil (≈4%), fiber (≈10%), starch (≈75%), and ash (≈2%). Therefore, maize has a wide range of both food and non-food applications (Ruan *et al.*, 2019). In this context, products can be obtained from immature and sweet maize to starches, flours, and semolinas, which are the base for bakery products, snacks, breakfast cereals, beer supplements, tortillas, and various other snacks.

An interesting industrial component is the oil contained in the germ of the maize kernel. This oil is in high demand, which has driven the development of specialized hybrid materials to increase content and optimize extraction. These maizes have an oil content greater than 6% and are obtained from hybrids specifically developed and selected for this purpose. The sought-after and recommended fatty acids are oleic or linoleic acid, palmitic, and stearic acids, which are associated with the reduction of arterial blockages (Darrah *et al.*, 2019).

On the other hand, the starch present in the endosperm of the grain is used as a base to produce maize syrup (Singh *et al.*, 2014), a sweetener in high demand by the beverage and soft drink industry (White, 2008), due to its sweetness comparable to sucrose, stability, and ease of use. The type of maize used for this purpose is hard maize, due to its starch and flour yield, as well as the quality and high purity obtained from it.

The starch from the endosperm of grain is also used by the distilling industry to produce alcoholic beverages and biofuels such as bioethanol (Ruan *et al.*, 2019). For this, the material is conditioned and hydrolyzed by yeasts to ultimately be distilled and recover the alcohol. During this process, high-nutritional-value by-products are generated, consisting of grain residues enriched during fermentation with the addition of biomass, vitamins, and amino acids, which are specifically used for the livestock sector.

Technological advancements and genetic improvements are closely linked to the specialization of maize cultivation, particularly in the focus on grain intended to produce a variety of products such as flours, starches, oils, protein, fiber, and maize semolina (Ruan *et al.*, 2019). These advances allow greater competitiveness and sustainability in maize cultivation, helping to meet the growing demand for maize-related products both nationally and globally.

This integrated approach to research and development is crucial for advancing food security and sustainability, as well as promoting economic prosperity in the agricultural sector. In this context, the specialization of maize cultivation in different products and specific uses allows its utilization according to the demands of specialized markets. In this regard, the selection of varieties and genetic improvement are of utmost importance to increase yield, tolerance to biotic and abiotic factors, and focus on specific applications, with the goal of contributing to the competitiveness and sustainability of the agricultural sector and the development of maize-producing regions.

These described examples highlight opportunities to diversify and revitalize maize production, which is a significant resource for farmers. The cultivation of specialty maize varieties meets specific market demands, offering greater profitability for this crop. Therefore, diversifying maize cultivation in Mexico represents an innovative and adaptive strategy in the agricultural production sector to address the changing needs of the consumer market.

CONCLUSIONS

Maize is a crop of significant global importance due to its use in human consumption, livestock feed, and various industries. In recent decades, there has been a notable increase in its demand, opening opportunities to enhance its performance and profitability. In Mexico, where challenges in maize production are prevalent, there is potential to encourage diversification of this crop through specialization. This involves promoting the production of varieties with specific uses, such as popcorn, pozole, and colored maize, which can offer higher profitability. Specialized maize production not only presents an alternative for meeting specific market niches but also serves as a means to diversify farmers' incomes and improve the profitability of maize production in Mexico. Identifying and promoting maize varieties with special uses can be a strategy to address the current challenges in maize production and advance towards food security and sovereignty for the country.

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Colegio de Postgraduados

Stenotrophomonas sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC: A Bacterial Consortium in the Production of *Zea mays* L. Under Different Agronomic Management Practices

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ABSTRACT

Objective: To evaluate the effect of the bacterial consortium *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN on three different agronomic management practices in maize (*Zea mays* L.) cultivation, with the question: Does the consortium of bacterial strains have a positive influence on maize production under different cultivation practices in the Ciénega region, Jalisco?

Design/methodology/approach: Treatments evaluated were TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. A randomized complete block design was established, and agronomic and yield variables were evaluated.

Results: The MT+MA treatment generated a 6.03% increase in grain yield; generated a 10.35% increase in ear height, a 4.87% decrease in plant height, and a 50% decrease in the consumption of synthetic products.

Limitations on study/implications: The agronomic management was carried out according to the practices of the region's farmers.

Findings/conclusions: The bacterial consortium *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN had a positive effect on maize cultivation for grain production, particularly when combined with agronomic management consisting of 50% traditional management+50% agroecological management. The bacterial consortium could be used as a bio-stimulant in maize production in the Ciénega region, Jalisco.

Keywords: *Zea mays* L., bioinoculants, agronomic management, bacterial consortium.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most nutritionally important cereals and, along with wheat and rice, is among the most widely cultivated and harvested crops worldwide (García

& Laval, 2019). Due to the domestication process and genetic improvement of maize, there is a need to use significant amounts of fertilizers to achieve acceptable yields (Martín & Ribera, 2015). Fertilization, particularly the application of mineral nitrogen to the crop, represents the highest cost in the production process (Zhao *et al.*, 2017). However, the irrational use of this input negatively impacts the agroecosystem (Baez-Rogelio *et al.*, 2017) and even human health (Vejan, 2016). Therefore, authors such as Armenta-Bojórquez *et al.* (2010) recommend optimizing the doses of nitrogen-based fertilizers without negatively affecting plant growth. Aguirre *et al.* (2009) suggest that an alternative to avoid the excessive use of nitrogen fertilizers in maize is the inoculation of seeds with plant growth-promoting bacterial strains (PGPB).

The production and application of inoculants formulated with various microbial species is a well-known practice in agriculture, though it is uncommonly used. Currently, within the framework of sustainability, the search for new microorganisms with diverse plant growth-promoting properties is an emerging research field, as these organisms can partially replace the use of pesticides and chemical fertilizers (Wang *et al.*, 2020). Many of these microorganisms originate from the rhizosphere, a zone of interaction between plant roots and soil, where plant roots exert influence through their exudates, and which harbors the highest population and diversity of microorganisms (Jacoby *et al.*, 2017). Furthermore, those microorganisms capable of enhancing crop development and yield through direct and indirect mechanisms are known as plant growth-promoting microorganisms (PGPM); direct mechanisms improve the nutritional status of the plant by increasing the exploration volume and functionality of roots, water uptake, nutrient availability and absorption, and the overall physiology of the plant (Kumar *et al.*, 2015). On the other hand, indirect mechanisms involve protection against stress caused by abiotic and biotic factors, including biological control against phytopathogens (Saraf *et al.*, 2014).

The application of microorganisms contributes to the ecological and sustainable management of agro-ecosystems. These microorganisms interact beneficially with the resident soil microbiota and enhance its adaptability to local climatic and agroecological conditions, making them suitable as inoculants to improve crop production (Cruz *et al.*, 2021). Pérez-Vázquez *et al.* (2018) consider agroecological production systems to be typically agro-diverse, resilient, energy-efficient, socially just, productive, and based on food sovereignty strategies that promote local production through family farming. These systems integrate innovation processes with a rational or zero use of synthetic inputs (fertilizers, pesticides), GMOs, hormones, and antibiotics in production. In this context, the objective of this study was to evaluate the effect of a bacterial consortium under three different agronomic management practices on maize grain production in the Ciénega region in the state of Jalisco.

MATERIALS AND METHODS

Location and Genetic Material: This research was conducted in a plot located in the community of La Víbora, municipality of Zapotlán del Rey, Jalisco, at coordinates N 20° 24’ 39.5” - W 102° 16’ 06.8”, during the spring/summer 2022 season. The bacterial consortium used consisted of the strains *Stenotrophomonas* sp. LIMN, *Enterobacter* sp.

LCMG, and *Rhizobium* sp. WFRFC, which were previously selected as plant growth-promoting rhizobacteria (Reséndiz *et al.*, 2022). Each strain was cultured on Tryptone Soy Agar (TSA) for 24 h, then grown in Tryptone Soy Broth with agitation at 100 rpm for 24 hours to reach a concentration of 1×10- colony-forming units (CFU)·ml^{-1}. Seeds of the commercial maize hybrid Pioneer P3095 were sown.

Experimental Design and Agronomic Management

The evaluation was established using a randomized complete block design with four replications. The agronomic management was carried out according to the practices of local farmers, with a row spacing of 0.85 meters. The plant spacing was 12 cm, and the experimental unit consisted of 12 rows of 20 linear meters each. The treatments used were: TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. The BC was applied three times during the growing season: at planting and two subsequent applications every 15 days. The three cultivation practices were related to nutrition, pest control, diseases, and weeds, in TM, synthetic inputs were used, TM+AM referred to reducing synthetic inputs by 50% and supplementing with 50% bioinputs (solid and liquid). AM utilized 100% bioinputs. The BC was diluted in water for application at a concentration of 1×10- CFU·ml^{-1} and applied via drench (without nozzle) using a manual pump.

Evaluated Variables

The response variables evaluated were: phenotypic variables including days to male flowering (DMF), days to female flowering (DFF) at the VT-R1 stage, as well as plant height (PH), ear height (EH), stem diameter (SD), ear diameter (ED), ear length (EL), measured in centimeters with data collected from four plants per treatment and block at the R1-R2 stage of the crop. Additionally, physiological readings were taken for maize plant vigor (VIGOR), which was measured five days after planting in each block and treatment. Samples were taken from a total length of three meters and reported as a percentage, and for yield (t ha^{-1}), three samples were taken from a length of three meters, considering three central rows per treatment and block, and adjusted to 14% grain moisture content.

Statistical Analysis

The data obtained for each of the studied variables were analyzed using SAS 9.4. An analysis of variance was performed, and significant statistical differences were assessed using Tukey's test ($P \leq 0.05$ and $P \leq 0.01$) for mean comparisons.

RESULTS AND DISCUSSION

Results. Under the three different agronomic management practices used for the crop, the phenotypic variables of days to male flowering (DMF), days to female flowering (DFF), plant height (PH), and ear height (EH), as well as the physiological variable of vigor (VIGOR), showed statistically significant differences between treatments and blocks (Table 1). The variables ear length (EL) and yield (YIELD) showed significant differences only between blocks (Table 1). Additionally, there were no significant differences (ns) in stem diameter (SD) and ear diameter (ED) between treatments and blocks (Table 1).

Considering the variables DMF and DFF, the ANOVA showed significant differences between treatments. The numerical differences of 0-2 days both between treatments and between the two flowering stages could have effects on subsequent stages. Notably, synchronization was observed between the two flowering stages in the TM+AM treatment, despite being the latest (Figure 1).

The phenotypic variable EH showed the highest value with the TM+AM treatment (145.75 cm, P≤0.05), while PH showed the highest value with the TM treatment (291.00 cm, P≤0.05) (Figure 2). The phenotypic variables EL, ED, and SD did not show differences between treatments (Figure 2).

Regarding plant physiological aspects, the VIGOR variable showed differences between the treatments under study, with the highest values observed in the MA treatment (P≤0.05) (Figure 3), followed by the TM and TM+AM treatments, with values of 75.33%, 74.33%, and 68.66%, respectively (Figure 3).

Another variable of interest is yield. Although it did not show statistically significant differences between treatments, a 6.03% increase (0.652 t ha^{-1}) was observed in the

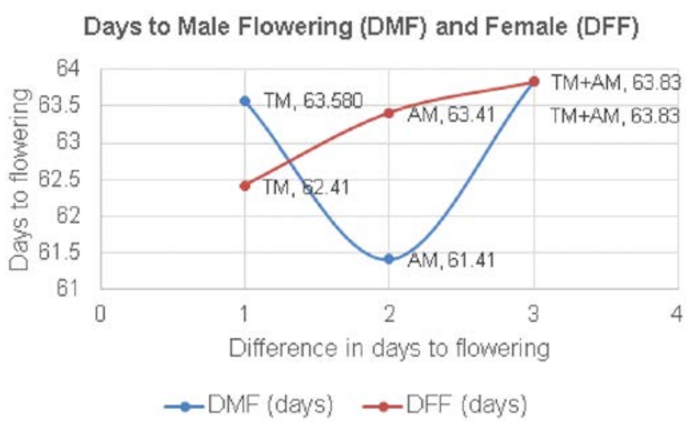

Figure 1. Comparison of means for the variables regarding treatments in yield and agronomic management in maize. TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC. DMF: Days to Male Flowering, DFF: Days to Female Flowering.

Table 1. Mean Squares and Significance of the Analysis of Variance for the Evaluated Variables with Respect to Blocks and Treatments.

S.V.	DF	DMF (days)	DFF (days)	PH (cm)	EH (cm)	SD (cm)	ED (cm)	EL (cm)	VIGOR (%)	YIELD (t ha^{-1})
TREAT	2	7.06*	2.12*	1434.24*	187.06*	0.005ns	0.02ns	0.08ns	51.69**	434281.36ns
BLOCKS	3	5.78*	0.81*	349.62*	53.60*	0.004ns	0.05ns	1.42*	97.76**	1748300.76*
E.E.		2.59	0.48	202.89	30.75	0.007	0.06	0.63	2.91	1291471.57
MEAN		62.94	63.22	273.77	139.08	2.40	3.39	15.78	72.77	11075.39
C.V.		2.55	1.10	5.20	3.98	3.70	7.34	5.05	2.34	10.26

**S.V.: Source of Variation, DF: Degrees of Freedom, TREAT: Treatments, BLOCK: Blocks, E.E.: Experimental Error, C.V.: Coefficient of Variation, DMF: Days to Male Flowering, DFF: Days to Female Flowering, PH: Plant Height, EH: Ear Height, SD: Stem Diameter, ED: Ear Diameter, EL: Ear Length, YIELD: Commercial Grain Yield. ns: Not Significant, *, **: Significant at P≤0.05 and P≤0.01, respectively.

TM+AM treatment (11.43 t ha^{-1}), compared to the TM and AM treatments, with values of 10.78 and 11.02 t ha^{-1}, respectively (Figure 4).

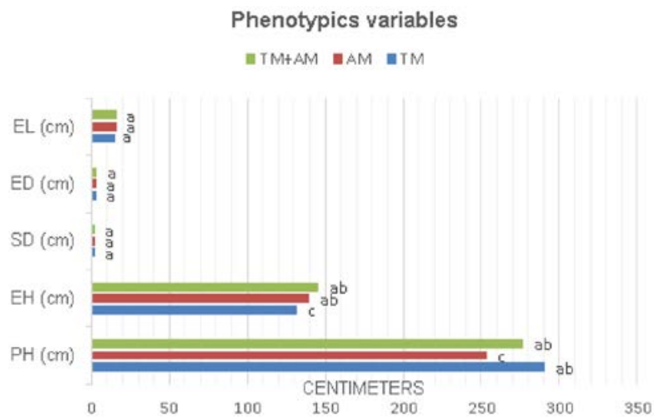

Figure 2. Comparison of means for phenotypic variables regarding agronomic management practices. PH=Plant Height, EH=Ear Height, SD=Stem Diameter, ED=Ear Diameter, EL=Ear Length. MT=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC.

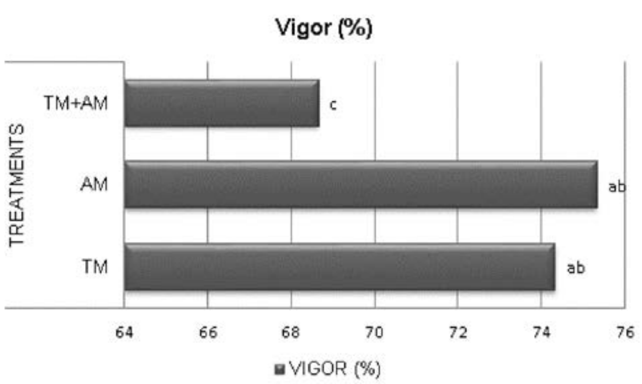

Figure 3. Comparison of mean values for treatments regarding VIGOR in maize. TM=100% traditional management+bacterial consortium (BC), TM+AM=50% traditional management+50% agroecological management+BC, and AM=100% agroecological management+BC.

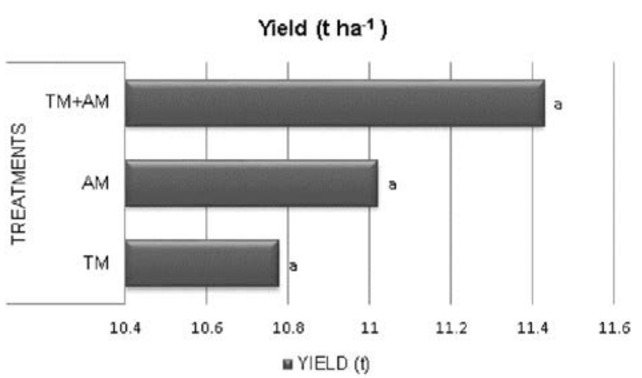

Figure 4. Comparison of mean grain yield across different agronomic management practices and its relation to the bacterial consortium in maize. TM = 100% traditional management + bacterial consortium (CB), TM+AM = 50% traditional management + 50% agroecological management + CB, and AM = 100% agroecological management + CB.

In this study, the effect of a bacterial consortium of three growth-promoting strains on maize grain production was analyzed, under three different agronomic management practices in the La Ciénega region, Jalisco. Previously, Barragán-Nava *et al.* (2022) isolated, identified, and characterized plant growth-promoting rhizobacteria from ten different land uses and planting cycles in the La Frailesca region, Chiapas, from which the strains *Stenotrophomonas* sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC were selected. Subsequently, Resendiz-Venado *et al.* (2022) evaluated their effect on maize seedling germination and growth, finding that the bacterial consortium increased the length, dry weight, and fresh weight of the plumule, as well as the number of roots. The field evaluation of the bacterial consortium conducted in this study confirms its potential as a bio-stimulant for maize cultivation.

The application of a bio-stimulant in the early vegetative stages of the crop was reflected in the vigor and reproductive stage; generally, the microorganisms in a bio-stimulant interact with the native microorganisms of the rhizosphere, the soil, and the genetic constitution of the maize type. In this regard, Lopes *et al.* (2016) mention that differences in the structure and composition of the rhizobacterial community are due to the selection performed by the rhizosphere from the microorganisms inhabiting the soil and plant, which modifies the abundance of functional groups according to their ability to adapt to rhizosphere conditions, thus shaping its bacterial community (Mendes *et al.*, 2014). In general, the rhizosphere is an environment regulated by a mixture of complex interactions between plants and microorganisms, where structural and functional diversity, as well as the stability of microbial communities, strongly influence crop quality (Ngullie *et al.*, 2015); that is, the rhizosphere creates a dynamic and nutrient-rich environment around the roots and maintains specific bacterial populations involved in activities that ensure crop stability and productivity (Dennis *et al.*, 2010). The rhizosphere is considered a selective pressure environment for horizontal gene transfer (HGT) events and is regarded as an important factor in increasing genetic diversity and, consequently, in bacterial evolution (Nemergut *et al.*, 2004).

The application of the bacterial consortium under the three agronomic managements favored the yield, showing an increase of 14.80% - 21.73% compared to the average yield reported for the La Ciénega region of 9.39 $t \cdot ha^{-1}$ (SIAP, 2022). Unlike its behavior between treatments where there was no statistically significant effect on this variable; however, a positive effect was observed with an increase of 2.23 and 6.03% in the yield of the AM and TM+AM treatments, respectively. Additionally, considering the reduction of 100% and 50% in the application of synthetic fertilizers, the profitability (data not shown) is improved in the AM and TM+AM agronomic managements. Additionally, considering the reduction of 100% and 50% in the application of synthetic fertilizers, profitability (data not shown) improved in the AM and TM+AM agronomic managements. Similar data were reported by Dotto *et al.* (2010), where the inoculation of *Herbaspirillum seropedicae* in the AS1570 hybrid did not significantly influence its productivity, but it was observed that the hybrid responded positively with an 8.6% increase in grain production. Bio-stimulants are bioproducts developed from one or several microbial strains; currently, their application is considered an appropriate method for introducing probiotics into agricultural soils (Yadav *et al.*, 2017); which release inorganic nutrients for plants from soil minerals, improve the structure of both the subsoil and the topsoil, increase water infiltration, enhance crop quality, and make plants more resistant to various pests and pathogenic organisms (Srivastava and Ngullie, 2009). Biofertilizers also increase soil microbial biodiversity by breaking the dormancy of microbial banks, due to a reinforcement of the relationship between biodiversity and ecosystem functioning (Bhardwaj *et al.*, 2014; Kulasooriya and Seneviratne, 2013). This increase in biodiversity strengthens soil health and enhances tolerance to stress caused by abiotic and biotic factors.

In this context, Santoyo *et al.* (2021) mentions that Plant Growth-Promoting Microorganisms (PGPM) refers to all microorganisms (bacteria, actinomycetes, fungi, or algae) that act through various mechanisms to enhance fertilization, phyto-stimulation, or disease suppression. They play an important role in sustainable agriculture, promote diversity and interaction with other beneficial microorganisms, and generally maintain the sustainability of systems. Various strains of the genera *Stenotrophomonas*, *Enterobacter*, and *Rhizobium* are known as PGPM in different crops, actively participating in biogeochemical nutrient cycles, mainly nitrogen and phosphorus, synthesizing antibiotics, among other characteristics, which support plant establishment, nutrition, and development (Goswami *et al.*, 2016; Shafi *et al.*, 2017). These characteristics exert various effects on the results obtained in the TM+AM and AM treatments, compared to the TM control, both in the phenotypic and physiological variables evaluated. For example, they influenced the variables of vigor, days to both male and female flowering, and the growth and development of the crop, positively affecting the reproductive stage. Sánchez-Yáñez *et al.* (2014) mention that a 50% reduction in nitrogen fertilizer generated a positive response in days to flowering and plant height in maize inoculated with plant growth-promoting bacteria; this suggests that these PGPM genera transformed maize root exudates into plant growth-promoting substances, which in turn induced increased stem growth. João *et al.* (2021) mention that inoculation with a *Bacillus* strain increased plant height and dry weight of shoot and root, changes attributed to the relatively increased abundance of strains from the Burkholderiaceae, Pseudomonadaceae, and Rhizobiaceae families, which are widely described as plant growth-promoting (García-Fraile *et al.*, 2012; Suárez-Moreno *et al.*, 2012; Redondo-Nieto *et al.*, 2013).

The consortium consisting of the *Stenotrophomonas* sp. LIMN, *Enterobacter* sp. LCMG, and *Rhizobium* sp. WFRFC strains also established an association with the evaluated maize hybrid plants, which is why they had a positive effect on the assessed variables. As reported, the plant genotype is a determining factor that directly influences the specificity of the bacteria-plant association, allowing the benefits of the inoculated strains to be obtained (Moreira, 2014).

CONCLUSIONS

The development of biotechnological tools that can be applied in the agroecological management of crops contributes to the sustainable use of genetic resources and the development of sustainable agriculture. Specifically, for maize cultivation, which requires a large amount of inputs for its production, such tools are highly relevant for reducing the environmental, economic, and agronomic impact generated. The application and use of a bacterial consortium consisting of *Enterobacter* sp. LCMG, *Rhizobium* sp. WFRFC, and *Stenotrophomonas* sp. LIMN, in combination with different agronomic management practices in maize cultivation was positive, achieving a 50% reduction in synthetic inputs with the traditional+agroecological management (TM+AM) treatment. Evidence is provided that under agroecological management, a 100% response is obtained compared to traditional (TM) or synthetic management, with a significant impact mainly on the variables DMF, DFF, VIGOR, and YIELD. The use of bacterial consortia and agroecological management reduces the long-term environmental and economic impact on agricultural crops.

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Colegio de Postgraduados

Sublethal Effects on the Biological Development of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) When Consuming Neem

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ABSTRACT

Objective: The Fall armyworm *S. frugiperda* was bred in laboratory and put under dose of watery extracts and oil of seed of *A. indica* with the aim of determining and to quantify the alterations in the biological development of larvae and pupae of caused by the consumption of *A. indica* and the doses in which the alterations happen.

Design/Methodology/Approach: Two experiments were development. In one eight doses were evaluated, with watery extract of seed of *A. indica* in 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04 and 0.01%. In the second the doses of oil of *A. indica* were evaluated 0.16, 0.09, 0.02, 0.016, 0.009, 0.002, 0.0016 and 0.0009%. In both experiments the variables were quantified: duration of the stage of larvae and pupae, survival of the larval stage and pupae, survival of the larva stage and pupae and weight of pupae.

Results: The larvae prolonged their development in three days more than the witness when consuming watery extract of *A. indica*; They reduced its survival like la rvae and pupa in 18 and 27.5% respectively. In Addition, was reduced the weight of pupas. When consuming oil of *A. indica*, the larvae delayed their development from three to 15 and 19 days more than the witness.

Study limitations/Implications: The study was carried out under laboratory conditions and in a single cycle; it is desirable to repeat the study for more cycles and with different populations of fall armyworm.

Finding/Conclusions: The adverse effects in larvae were pronounced of three forms: 1) the duration of the larval stage altered when staying the larvae by more days in that stage without changing pupae. 2) the survival of the larval stage was reduced and 3) it decreased weight of pupa.

Keywords: Inhibition of growth, watery extracts, oil of *A. indica*, survival of larvae.

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INTRODUCTION

The most important pest insect for maize cultivation is the fall armyworm (*Spodoptera frugiperda* Smith) (Ngegba *et al.*, 2022). Various control methods are used to manage this pest, including chemical, rational, and neem (*Azadirachta indica*) (Silva *et al.*, 2015; Ewansiha *et al.*, 2023). Neem causes mortality in more than 400 evaluated pest insect species (Schmutterer and Rembold 1995; Ahissou *et al.*, 2022). Ingestion of this substance in insects results in inhibition of feeding and growth, as well as repellency, reduction in oviposition, sterilization, and changes in the neuroendocrine system, leading to suppression of ecdysis (Dorn 1996; Roel *et al.*, 2010; De Campos *et al.*, 2014; Pérez-Cogollo *et al.*, 2015; and Figueroa Gualteros *et al.*, 2019). Various studies have been conducted to understand the effects of neem on the physiological and behavioral alterations across multiple insect species (Schmutterer and Rembold 1995; De Oliveira *et al.*, 2010; Hakeem *et al.*, 2018; Moonga *et al.*, 2018; Mwanauta *et al.*, 2021).

In larvae of *Trichoplusia ni* (Hübner) and *Spodoptera exigua* (Hübner), the developmental period and larval mortality were prolonged upon ingesting *A. indica* extracts (Prabhaker *et al.*, 1986), as well as growth inhibition in first-instar larvae of *S. frugiperda* (Jacobson *et al.*, 1984). In *S. frugiperda*, the efficacy of neem in causing mortality has been studied (Phambala *et al.*, 2020; Ahissou *et al.*, 2022; Ngegba *et al.*, 2022; Chan *et al.*, 2023) and general symptoms caused by the ingestion of *A. indica* have been documented (De Oliveira *et al.*, 2010). However, the effects of consumption and the alterations in the biology of larvae and pupae have not been determinated.

The present study was conducted to determine and quantify the alterations in the biological development of *S. frugiperda* larvae and pupae caused by the consumption of neem, as well as the levels of ingestion at which these alterations occur.

MATERIALS AND METHODS

Aqueous extracts and seed oil of *A. indica* were evaluated in an artificial diet against *S. frugiperda* at a temperature of 25±2 °C, relative humidity of 60±10%, in the Entomology Laboratory of the Maize Program at the International Maize and Wheat Improvement Center (CIMMYT) in El Batán, Texcoco, State of Mexico.

To conduct the experiments, it was necessary to have a population of corn earworm larvae for artificial infestation. The first-instar larvae of *S. frugiperda* were obtained from the mass rearing of insects at the Entomology Laboratory of CIMMYT.

The aqueous extracts were obtained from ground seeds of *A. indica* from maize plantations in the municipality of San Pedro Tututepec, on the Oaxaca Coast, Oaxaca. The *A. indica* oil, NEEM OIL EXTRACT® at 93% with a concentration of azadirachtin of 4,463 ppm, was recommended at a dose of 250 ml in 200 liters of water. To determine the doses evaluated in the experiments of this study, 2 preliminary bioassays were conducted with generalized ranges of the concentration of *A. indica*, evaluating the same variables presented here, until the doses to be evaluated in these treatments were determined.

Experiment with Aqueous Extract of *A. indica*

A laboratory experiment was conducted using eight doses of *A. indica* seed aqueous extract at concentrations of 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01%. To prepare the different doses, 4.0 g of *A. indica* seed was mixed with 100 ml of water 24 hours before preparing the diet. The doses were kept in an amber bottle to allow for better extraction and preservation of water-soluble compounds. After 24 hours, the solid portion was separated from the liquid by filtering through a sieve. Once the artificial diet was prepared, the obtained liquid compound was added. This way, the indicated doses were prepared, along with a control to which only 100 ml of water was added.

Experiment with *A. indica* Oil

In the *A. indica* oil experiment, the following doses were applied: 0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, and 0.00088 ml (equivalent to 0.16, 0.09, 0.02, 0.016, 0.009, 0.002, 0.0016, and 0.0009%). In each of the doses, the oil was measured with a 200 μl Gilson pipette and mixed with water to obtain a volume of 100 ml for each treatment. The artificial diet described by Mihm (1983) was used for these tests, into which the aqueous extracts at 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01% were incorporated during its preparation, resulting in approximately 600 g of diet for each treatment. The amount of water required for preparing the extract was included in the total amount of water normally used for preparing the artificial diet. In addition to the corresponding diet, a control diet was also prepared for each dose, to which only water was added. After preparation, the diets were poured into glass tubes with a capacity of 5 cm in height × 1.5 cm in diameter. Twenty-four hours after the preparation of the artificial diet with *A. indica* at different concentrations, first-instar *S. frugiperda* larvae were placed in the glass tubes. There were 100 tubes for each dose, with one newly hatched larva placed in each tube. Each tube was covered with sterilized cotton and kept in a rearing chamber at 25±2 °C and 60±10% relative humidity. The 100 larvae per dose were checked daily to quantify mortality and the duration of the larval stage until transformation to the pupal stage.

The pupae were weighed at 24 hours using an analytical scale with a precision of 0.001 g and were transferred to other tubes, where they remained until the emergence of adults. For each dose, the duration and survival of the larval and pupal stages, as well as the weight of the pupae, were evaluated. Larval survival is the percentage of larvae that progress to the pupal stage, and pupal survival is the percentage of pupae that emerge as adults. In the nine doses evaluated, eight concentrations of *A. indica* oil (0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, 0.00088 ml made up to 100 ml with water) and a control without *A. indica* were included. The procedure and variables evaluated were the same as those used for the experiments with aqueous extracts of *A. indica* seeds.

For conducting these tests, the artificial diet described by Mihm (1983) was used, into which the aqueous extracts at 4.0, 1.0, 0.7, 0.4, 0.1, 0.07, 0.04, and 0.01% were incorporated during its preparation, resulting in approximately 600 g of diet for each treatment. The amount of water required for preparing the extract was included in the total amount of water normally used for preparing the artificial diet. In addition to the diet corresponding to each dose, a control diet was also prepared, to which only water was added. After preparation, the diets were poured into glass tubes with a capacity of 5 cm in height × 1.5 cm in diameter.

Twenty-four hours after preparing the artificial diet with *A. indica* at different concentrations, first-instar *S. frugiperda* larvae were placed in the glass tubes. One hundred tubes were prepared for each dose, with one newly hatched larva placed in each tube. Each tube was covered with sterilized cotton and kept in a rearing chamber at 25±2 °C and 60±10% relative humidity. The 100 larvae per dose were checked daily to quantify mortality and the duration of the larval stage until transformation to the pupal stage.

The pupae were weighed at 24 hours and transferred to other tubes, where they remained until the emergence of adults. For each dose, the duration and survival of the larval and pupal stages, as well as the weight of the pupae, were evaluated. Larval survival is the percentage of larvae that progress to the pupal stage, and pupal survival is the percentage of pupae that emerge as adults.

In the nine doses evaluated (eight concentrations of *A. indica* oil: 0.154, 0.088, 0.022, 0.0154, 0.0088, 0.0022, 0.00154, 0.00088 ml rated at 100 ml with water, and a control without *A. indica*), the procedure and variables evaluated were the same as those used for the experiments with aqueous extracts of *A. indica* seeds.

Statistical Analysis

The obtained data were subjected to an analysis of variance and analyzed using the non-parametric rank test (PROC RANKS) (SAS 2002). Each treatment had 5 repetitions, and each repetition consisted of a batch of 20 larvae. For both experiments in this stage, the following variables were evaluated: duration of the larval stage, duration of the pupal stage, larval and pupal survival, and pupal weight. These variables are suitable for describing the developmental stages of *S. frugiperda* (Figueroa Gualteros *et al.*, 2019; Phambala *et al.*, 2020).

RESULTS

Experiment with Aqueous Extract of *A. indica*

In all treatments, the larvae fed on the provided diet. The concentrations of *A. indica* aqueous extracts at 0.4, 0.7, 1, and 4% added to the artificial diet resulted in 100% mortality in *S. frugiperda* larvae. This analysis presents the sublethal effects of *A. indica* on the larval and pupal development of the insect.

In the experiment with aqueous extracts, for the variable of larval stage duration, all concentrations were significantly different from the control, except for the 0.01% concentration ($F_{4, 76}$=0.526; P<0.0621) (Figure 1). In contrast, there were no statistically significant differences between treatments for the variable of pupal stage duration in days ($F_{4, 76}$=7.407; P<0.023) (Figure 1).

Larval survival was statistically different among treatments (Figure 2). This significant difference occurred between the 0.04% concentration and the control ($F_{4, 76}$=1.9437; P <0.111). Pupal survival was significantly different among the various concentrations ($F_{4, 76}$=2.9903; P=0.024), with the 0.1% aqueous extract concentration showing the most pronounced effect, reducing survival by 27.5% compared to the control. Regarding pupal

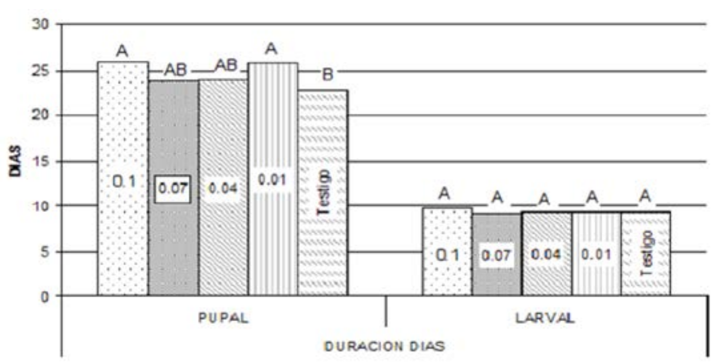

Figure 1. Duration in days of the larval and pupal stages, and pupal weight of *S. frugiperda*, reared on artificial diet mixed with aqueous extracts of *A. indica* seeds. Means followed by the same letter in the columns do not differ from each other, according to the rank test at P<0.05.

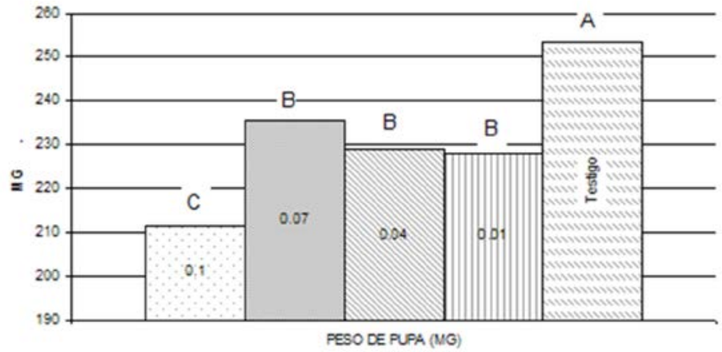

Figure 2. Pupal weight of *S. frugiperda*, reared on artificial diet mixed with aqueous extracts of *A. indica* seeds. Means followed by the same letter in the columns do not differ from each other, according to the rank test at P<0.05.

weight, there are statistical differences between the treatments and the control, with the 0.1% aqueous extract concentration standing out (Figure 2).

Experiment with *A. indica* Seed Oil

For the variable of larval stage duration, all treatments with different doses of *A. indica* oil were not significantly different from the control, except for the treatment with the 0.09 concentration of *A. indica* oil ($F_{7, 133}$=5.44; P=0.06317) (Figure 3). However, there were statistical differences among all treatments with different concentrations of *A. indica* oil and the control for the variable of pupal stage duration, except for the treatment with the 0.002 concentration, which was similar to the control ($F_{7, 133}$=7.538; P=0.04287) (Figure 3).

Larval survival was significantly different among the treatments with the concentrations of 0.02, 0.009, and 0.0016% compared to the control ($F_{7, 133}$=13.018; P=0.03128) (Figure 4). For pupal survival, statistical differences were observed among the treatments with concentrations of 0.16, 0.09, and 0.009, while the other treatments were similar to the control ($F_{7, 133}$=12.1411; P=0.02872) (Figure 4). In the variable of pupal weight, all treatments with different concentrations of *A. indica* oil were statistically different from the control ($F_{7, 133}$=35.2; P<0.01947) (Figure 5).

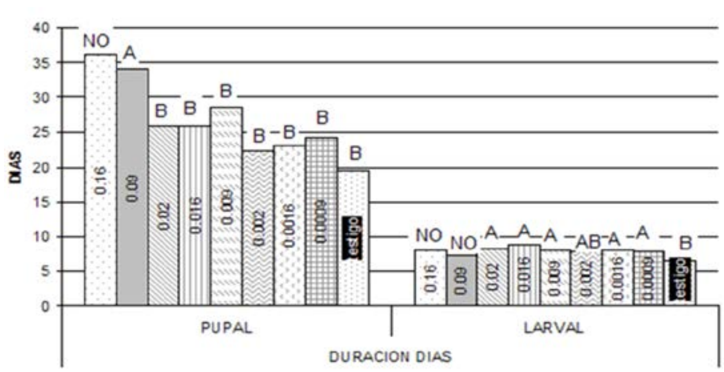

Figure 3. Duration of the larval and pupal stages of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

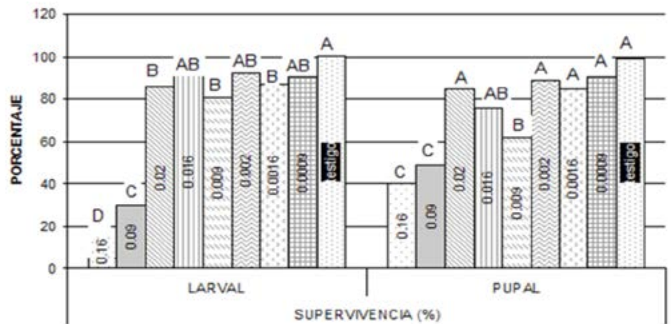

Figure 4. Survival of the larval and pupal stages of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

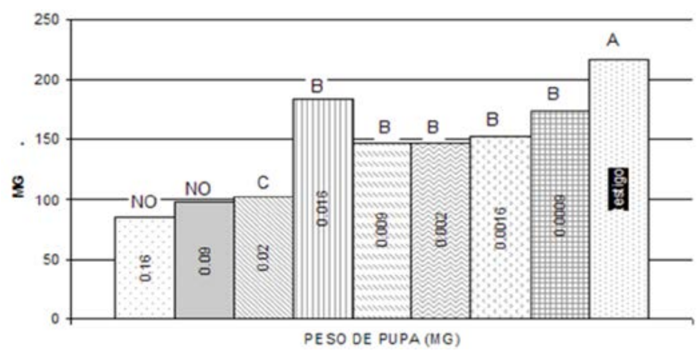

Figure 5. Pupal weight of *S. frugiperda*, fed on artificial diet with *A. indica* oil. Means followed by the same letter in the columns do not differ from each other according to the rank test at P<0.05.

DISCUSSION

Experiment with Aqueous Extracts of *A. indica*

When *S. frugiperda* larvae consumed diet with aqueous extracts of *A. indica* at the concentrations of 0.4, 0.7, 1, and 4%, there was 100% mortality. This effect is significant as it can reduce the number of individuals and/or the population of the pest due to ingestion poisoning, which is consistent with observations made in *Spodoptera littoralis* (Pineda *et al.*, 2004a; Pineda *et al.*, 2006b; Schneider *et al.*, 2004).

Larvae fed with 0.1% and 0.01% aqueous extract of *A. indica* mixed in artificial diet only showed sublethal effects, lasting 3.1 and 3.0 days, respectively, longer than the control in the larval stage (see Figure 1). This effect is likely related to the neurotoxic action of natural insecticides, which causes paralysis in intoxicated insects; these insects stop feeding and grow slowly (Pineda *et al.*, 2006).

Survival at the different concentrations of aqueous extracts of *A. indica* mixed with artificial diet was similar to the control, except at the 0.04% concentration, which recorded a 16.6% lower survival rate of the larval stage compared to the control larvae (Figure 2). Similar results were obtained by Figueroa Gualteros *et al.* (2019) and Duarte *et al.* (2019). Pupal survival was different among all treatments fed on artificial diet with various concentrations of aqueous extracts of *A. indica*, with the most notable effect at the 0.1% concentration, which reduced survival by 27.5% compared to the control. These data are consistent with Prabhaker *et al.* (1986), who found that incorporating *A. indica* seed extracts into artificial diet at concentrations of 0.02%, 0.2%, and 2.0% prolonged development and induced mortality in all larval stages of *Spodoptera exigua* (Hubner), preventing pupation. Regarding pupal weight, all concentrations differed from the treatment with artificial diet without aqueous extract of *A. indica*, which had the highest pupal weight. The 0.1% aqueous extract concentration was significantly the lowest, reducing the average pupal weight by 18.55% compared to the control (Figure 3). This finding is consistent with the data observed by Prabhaker *et al.* (1986) in *Spodoptera exigua* (Hübner) larvae, who noted that reduced feeding led to a decrease in pupal weight (PP) compared to control larvae.

Experiment with *A. indica* Seed Oil

When analyzing the duration of the larval stage, treatments with the concentrations 0.09, 0.009, and 0.0009 of *A. indica* oil mixed in artificial diet had 14.6, 9.2, and 15 days longer duration than the control. This effect is caused by intoxication or inhibition of feeding on the artificial diet containing substances unpleasant to the insect; reactions observed in intoxicated insects, which stop feeding and grow slowly (Pineda *et al.*, 2006).

Pupal duration between the control and the different treatments varied, except in the 0.002% treatment, which was the same. The 0.016% concentration stood out with 2.27 days longer duration, possibly due to interference with the neuroendocrine system by affecting ecdysone and juvenile hormone synthesis (Schmutterer, 1988).

Larval survival was reduced by 95% at the 0.16% concentration and by 70% at the 0.09% concentration of *A. indica* seed oil (Figure 5) compared to the larval survival in the control. Pupal survival showed the most significant reductions at the 0.16% and 0.09% concentrations, with reductions of 59% and 50.3%, respectively, also caused by insect intoxication. This toxic effect was demonstrated in the study conducted by Chan *et al.* (2023). In contrast, pupal weight decreased in all *A. indica* oil treatments compared to the pupal weight in the control, with the 0.16% and 0.09% oil treatments showing the greatest reductions at 60.9% and 55.17%, respectively. This could be a consequence of the effects of consuming artificial diet with *A. indica*, as reported by various researchers in other insect species (Gaaboub and Hayes 1984; Koul *et al.*, 1990; Dorn, 1996; Mitchell *et al.*, 2004; García *et al.*, 2006; Mwanauta *et al.*, 2021).

The results of both experiments (with aqueous extract and with seed oil) demonstrated that the best effects were achieved when using aqueous extracts rather than seed oil of *A. indica*. This may be because aqueous extracts contain other secondary compounds in addition to azadirachtin (Opender, 2004), which are removed during the oil extraction process and discarded with the rest of the seed, or perhaps because the aqueous extract can penetrate or be ingested more easily into the insect's body than the oil.

CONCLUSIONS

Sublethal effects on *S. frugiperda* larvae fed artificial diet mixed with different concentrations of aqueous extracts or *A. indica* oil were observed in the following ways: the duration of the larval stage was altered as larvae remained in this phase for more days. Survival of the larval stage was reduced because it prevented all larvae from pupating; in other words, a portion of the insect population was killed. The 0.4% concentration of *A.*

indica aqueous extract and higher concentrations in artificial diet caused 100% mortality in *S. frugiperda* larvae. It was found that the 0.1% treatment extended the duration of the larval stage by 3.2 days, reduced larval survival by 18%, and pupal survival by 27.5% compared to the control. There was an increase in the duration of the larval stage of up to 86.6% and 75.3% when *S. frugiperda* was fed artificial diet with *A. indica* oil at 0.16% and 0.09% concentrations, and 21.2% and 12.1% in the duration of the pupal stage for the same treatments. Larvae of *S. frugiperda* fed with artificial diet mixed with the highest concentrations of *A. indica* oil showed a longer duration of the larval stage and recorded lower weights, approximately 61% less than the control. The 1.0% and 0.4% aqueous extract treatments caused pupae to start halting their growth in the 5^{th} and 6^{th} instars, decreasing on average by 14% to 35%. The 0.5% *A. indica* oil treatment recorded 40% of pupae and 45% of larvae in the 6^{th} instar, and the 0.16% treatment had 58% of pupae and 23% in pre-pupa.

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Colegio de Postgraduados

Chemical Management of *Helianthus annuus* L. as a Broadleaf Weed in Interaction with the *Zea mays* L. Crop

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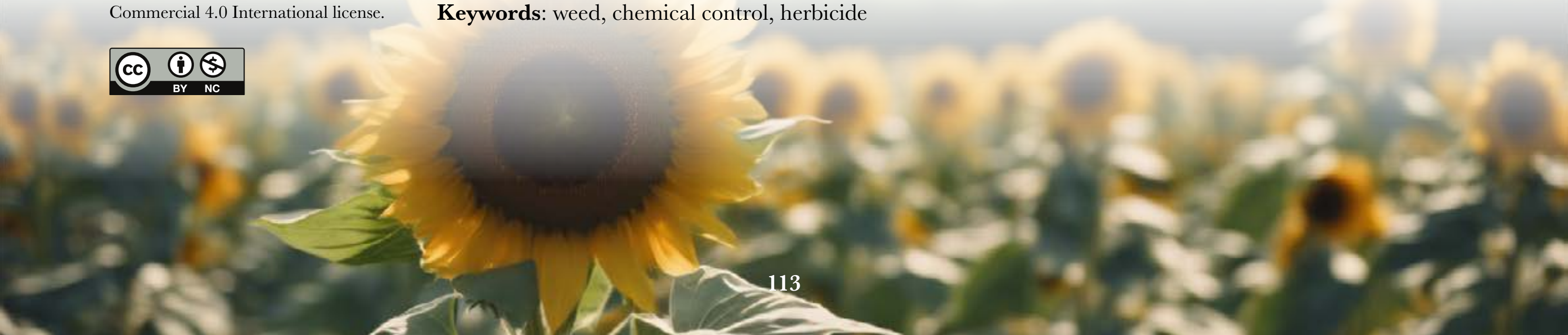
ABSTRACT

Objective: To evaluate the biological effectiveness of progressive doses of the herbicide Condraz® (tritosulfuron +dicamba) in post-emergence in dicotyledonous plants grown in consortium with corn in a greenhouse.
Design/methodology/approach: The research was performed out in a greenhouse within the facilities of the Colegio Superior Agropecuario del Estado de Guerrero. Dekalb 357® corn was established, planted in a consortium with sunflower, to measure the response to different dosages of the herbicide Condraz® (tritosulfuron+dicamba). We worked with a completely randomized block experimental design (DBCA).
Results: The treatments were 100% effective, because they eliminated the sunflower plants used as a representative of dicotyledonous weeds; while, in treatment T1 (water) the number of these was not affected, which was 10 sunflower plants per pot in the two experiments.
Limitations on study/implications: Our results are specific for the management of dicotyledonous weeds in corn.
Findings/conclusions: Condraz® herbicide is effective in eliminating dicotyledonous weeds when applied in doses ranging from 100 to 190 g per ha.

Keywords: weed, chemical control, herbicide



INTRODUCTION

Corn (*Zea mays* L.) is the most important cereal worldwide, surpassing rice and wheat in production volume (OECD/FAO, 2023). In 2022, global corn production reached 1,440 Mt, with Mexico producing 26.6 Mt, ranking sixth in the production of this cereal (FAOSTAT, 2024). In Mexico, corn is the most significant crop in terms of nutrition, gastronomy, and culture; it occupies more productive land than any other crop (De los Santos-Ramos *et al.*, 2017).

In Mexico, white corn production is primarily intended for human consumption; however, the country is not self-sufficient in supplying yellow corn, which is mainly imported from the United States (SAGARPA, 2017).

In every production cycle, there is the persistent issue of weed incidence, which directly affects the development of the corn plant. If left uncontrolled, weeds can lead to total crop loss (Sharma *et al.*, 2021). Weeds can be more harmful than any other pest (Idziak *et al.*, 2022); they actively compete for sunlight, water, nutrients, and space, causing damage to the phenology and morphology of the corn plant, thus affecting crop development and yield, resulting in economic losses (Sharma *et al.*, 2021).

There are cultural, chemical, mechanical, and biological methods for weed management (Rastgordani *et al.*, 2013), which are applied with the aim of suppressing the growth of weed flowers (Shahzad *et al.*, 2021). However, manual and cultural control methods require a significant amount of labor, which increases costs and is not always readily available (Perez-Ruiz *et al.*, 2013).

Currently, weed control in corn cultivation relies on the application of herbicides, which are effective and safe for ensuring crop development and yield (Waligóra *et al.*, 2012). The use of herbicides containing at least two active ingredients with different modes of action leads to control over a broad range of weed species, prevents damage by using lower herbicide doses, reduces residual effects in plants and soil, delays the onset of resistance, and lowers production costs (Idziak and Woznica, 2020). Due to these advantages, chemical control is the most effective and popular method for weed management (Martínez *et al.*, 2021). However, excessive use can contribute to the development of resistant species, which may affect crop quality or have an impact on the environment and health (Shahzad *et al.*, 2021). Globally, in 2022, 350 cases of glyphosate resistance were reported (Arispe-Vázquez *et al.*, 2023). Therefore, it is necessary to adopt strategies that help reduce this issue by using new molecules applied in optimal doses and at the appropriate time. Among the herbicides available in Mexico is Condraz® (tritosulfuron+dicamba), a systemic, post-emergence herbicide selective for controlling broadleaf weeds in corn and wheat crops; it exhibits rapid penetration into weeds, low residuality in the soil, minimal impact on the soil ecosystem, and is suitable for crop rotation (Çağlar *et al.*, 2023). The hypothesis of this study is that higher doses will have a greater control effect. Thus, the objective of the research was to evaluate the biological effectiveness of progressive doses of the herbicide Condraz® (tritosulfuron+dicamba) in post-emergence in dicotyledonous plants grown in consortium with corn in a greenhouse.

MATERIALS AND METHODS

Study Area

The research was conducted in a low-tech greenhouse located at the Superior Agricultural College of the State of Guerrero (CSAEGRO), situated at km 14.5 on the Iguala-Cocula road (18° 14' 00" N and 99° 40' 00" W, at an altitude of 640 m). This region has a warm sub-humid climate Awo (w) (i) g, with summer rains; the average annual precipitation and temperature are 797 mm and 26.4 °C, respectively (Ayvar *et al.*, 2021).

Genetic Material

The improved white corn hybrid Dekalb-357® (DK-357®) was used, which is a dual-purpose variety (grain and forage), adaptable to various regions, particularly tropical climates. The planting density was 50,000 to 60,000 seeds per ha^{-1}, with a germination and emergence rate exceeding 98%. The plant exhibits excellent foliar health, an average height of 250-260 cm, and 110 cm at the ear insertion; it begins flowering at 65-70 days and is harvested at 155-160 days. The ear coverage is good. The grain type is semi-dent and has 18 to 20 rows per ear (Reyna *et al.*, 2023). The sunflower (*Helianthus annuus*) variety Ekilore is an annual herbaceous plant with an average height of 2.5 meters, without branches. Its stem is hirsute, with alternate, large, ovate leaves with serrated margins. It has inflorescence in a large terminal capitulum, with yellow ligulate flowers on the exterior and dark florets in the center. The fruit is a grayish achene, usually with black bands (Girón, 2023).

Study Treatments

Corn Dekalb 357® was established in consortium with sunflower to measure the response to different doses of the herbicide Condraz® (tritosulfuron+dicamba). T1=water, T2=100 g per ha^{-1}, T3=115 g per ha^{-1}, T4=130 g per ha^{-1}, T5=155 g per ha^{-1}, T6=160 g per ha^{-1}, T7=175 g per ha^{-1}, and T8=190 g per ha^{-1}. Sunflower plants were included as a representative species of dicotyledonous weeds to assess the toxicity of the chemical molecules contained in this product.

Experimental Design and Units

The eight treatments, each with five replications, were arranged in a completely randomized block design (CRBD); a total of 40 experimental units were used. Each unit consisted of a black polyethylene pot with dimensions 11×11×15 cm, filled with 1.5 kg of substrate, one corn plant, and ten sunflower plants to recreate aspects of weed-crop interference, *i.e.*, to promote interspecific competition. The experiment was established in duplicate.

Greenhouse Characteristics

The crop was established in a bicentennial type of greenhouse with a white, waterproof plastic cover, with a thickness of 0.125 mm, tensile strength of 49 N, and puncture resistance of 62 N. The cover reflects 20 to 30% and absorbs between 70 and 80% of solar light (Reyna *et al.*, 2023).

Preparation of the Substrate and Pots

The substrate was prepared by mixing forest soil and sifted sand in a 1:1 ratio. A total of 80 pots (40 per experiment) were filled with this mixture. The pots were then weighed to standardize the substrate content to 1.5 kg per experimental unit.

Planting, Irrigation, and Fertilization

Before direct planting, the pots were watered to field capacity to provide the seeds with the appropriate conditions for emergence. Four corn seeds and 15 sunflower seeds were sown per experimental unit. Twenty-four hours after planting, manual watering was performed each morning to field capacity to keep the substrate moist and prevent water stress. Five grams of diammonium phosphate (DAP) (18-46-00) were applied per pot 15 days after planting (DAPL).

Application of Condraz® Doses

The herbicide was applied 21 days after planting (DAPL). The dose of the herbicide for each treatment was diluted in 350 mL of water. Manual spraying was carried out using a 500 mL plastic sprayer, where the liquid was shaken to homogenize it and then sprayed onto the foliage. The total spray volume was 2.0 mL per experimental unit, with only one application of the treatments being performed.

Study Variables

At the end of the experiment (36 DAPL), the study variables were measured, except for the plant's foliar coverage, which was evaluated three days after the application of the treatments.

Effectiveness Percentage

The number of surviving sunflower plants in each treatment was counted and then divided by the number of weeds present in the control (water). The result was multiplied by 100 to obtain the effectiveness percentage. The results were interpreted using the scale provided by the European Weed Research Society (EWRS) (Champion, 2000) (Table 1).

Table 1. Scale proposed by the European Weed Research Society (EWRS) to evaluate weed control.

Worth	Weed Control (%)	Effect on weeds
1	99.0 - 100.0	Deat
2	96.5 - 99.0	Very good control
3	93.0 - 96.5	Good control
4	87.5 - 93.0	Control sufficient
5	80.0 - 87.5	Control medium
6	70.0 - 80.0	Control regular
7	50.0 - 70.0	Control poor
8	1.0 - 50.0	Control very poor
9	0.0 - 1.0	Without effect

Phytotoxicity percentage. At the end of the experiment, the color tone of the plants in the control treatment (without application) was observed and compared with that of the plants in the other treatments.

Percentage of foliar coverage of corn and sunflower plants. A photograph was taken of the plants in each experimental unit (40 images), and the digital mobile application (Canopeo), developed using Matlab (Matrix Laboratory), was used to obtain foliar coverage. Measurement of this variable began three days after the treatments were applied, with four evaluations conducted at three-day intervals.

Height of corn plants (cm). The height was measured in centimeters, from the base of the stem to the apex of the last leaf.

Number of leaves per corn plant. Fully expanded leaves per plant were counted.

Fresh plant weight (corn and sunflower). Corn and sunflower plants were removed from the substrate and weighed using a digital scale. The data were recorded separately by species, treatment, and replication.

Dry plant weight (corn and sunflower). Corn and sunflower plants were separately placed in labeled paper bags and dried in an oven at 70 °C for 72 hours; they were then weighed on a digital scale independently.

Statistical analysis. An analysis of variance and multiple mean comparison test were performed using the Tukey method (α=0.05). Additionally, a linear regression and correlation analysis were conducted using SAS software (SAS Institute Inc., 2020).

Effectiveness Percentage. This variable showed highly significant differences because of herbicide application in both Experiment 1 (Pr>F=0.0001**) and Experiment 2 (Pr>F=0.0001**). It was determined that in both experiments, all treatments exhibited 100% effectiveness, as they eliminated the sunflower plants used as representatives of broadleaf weeds. Meanwhile, treatment T1 (water) did not affect the number of these plants, with 10 sunflower plants per pot in both experiments. The results suggest that the herbicide Condraz® effectively eliminates dicotyledonous weeds when applied at doses ranging from 100 to 190 g per ha^{-1}.

These results are similar to those of Tamayo *et al.* (2020), who achieved 92.5% effectiveness with the herbicide Condraz® for controlling broadleaf weeds 30 days after application (DAA) at a dose of 250 g per ha^{-1}, which is 150.0 to 131.6% higher than the dose used in the present study. Additionally, Çağlar *et al.* (2023) applied Condraz® at doses of 200 to 250 g per ha^{-1} at 21 DAA, achieving 85% effectiveness in controlling weed species such as *Chenopodium album* L., *Setaria verticillata* (L.) P. Beauv., and *Abutilon theophrasti* Medicus in corn cultivation. These effectiveness rates are lower than those obtained in the present study with lower concentrations in the applications.

Percentage of Phytotoxicity in Corn Plants

This variable did not show significant effects due to the application of treatments in Experiments 1 (Pr>F=0.4520NS) and 2 (Pr>F=0.4520NS). It was found that applications of Condraz® (tritosulfuron+dicamba) at the seven evaluated doses did not induce phytotoxic symptoms in corn plants DK 357®. These results align with those obtained by Tamayo *et al.* (2020), who determined that treatment with Condraz® at concentrations of 200 to 250 g per ha^{-1} in corn for the control of broadleaf weeds did not cause negative effects on the crop. The results agree with these authors, as it was confirmed that applications of the mentioned doses do not cause phytotoxicity problems in corn crops, both 7 and 15 DAA.

Corn Plant Height

The treatments caused significant differences in the average values of this variable, in Experiment 1 (Pr>F=0.0500*) and Experiment 2 (Pr>F=0.0442*). In Experiment 1, it was observed that the treatment with 160 g per ha^{-1} of Condraz® (T6) resulted in plants with greater height (average of 22.5 cm); however, it was only statistically different from the treatments with doses of 100 (T2) and 0 (T1) g per ha^{-1}, surpassing them by 18.7% and 8.4%, respectively (Table 2).

In Experiment 2, the treatments (T2) and (T7) with doses of 100 and 175 g per ha^{-1} of Condraz®, respectively, resulted in plants with the greatest height (average of 21.5 cm). However, no statistical difference was observed compared to the other treatments, including the control (T1); where the averages ranged between 17.3 cm (T1) and 21.3 cm (T6) (Table 2). According to these results, it is suggested that applying Condraz® at different doses can promote an increase in corn plant height, as it eliminates weed competition 36 days after emergence.

These results are consistent with those reported by Çağlar *et al.* (2023), who applied Condraz® (tritosulfuron+dicamba) at a dose of 250 g per ha^{-1} in corn crops for broadleaf weed control. They also noted that in the herbicide-treated plants, the maximum corn plant height was 27 cm, whereas the average height was 16 cm in the control, 21 DAA. Authors such as Martínez *et al.* (2021) mention that in weed-free conditions from the beginning of planting up to 10 days after emergence (dde), corn plants exhibit greater height due to the lack of competition.

Table 2. Height and number of leaves per corn plant.

Treatment	Height of corn plant (cm)		Number of leaves per plant	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
T1	18.30 b†	17.30 ab	7.00 a	6.40 b
T2	20.60 b	21.50 a	7.20 a	7.20 ab
T3	22.00 ab	20.30 a	7.80 a	7.20 ab
T4	21.80 ab	20.70 ab	7.40 a	7.40 ab
T5	22.20 ab	20.80 ab	7.40 a	7.40 ab
T6	22.50 a	21.30 ab	7.80 a	7.40 ab
T7	20.70 ab	21.50 a	7.20 a	8.20 a
T8	21.70 ab	21.00 ab	7.40 a	7.80 ab
DMS	4.12	4.12	0.85	0.38
Prob. F	0.0500*	0.0500*	0.4373NS	0.0500*

† Values whith the same letters in the same column are not statistically diferent. DMH: Tukey's mínimum honest difference α=0.05.

Number of leaves per corn plant

This characteristic, related to the plant's photosynthetic area, was not significantly affected in Experiment 1 (Pr>F=0.4373NS), but showed significant variations in Experiment 2 (Pr>F=0.0500*). In Experiment 1, it was determined that although all treatments with Condraz® doses (T2 to T8) resulted in plants with greater height compared to the control plants (T1), the differences in the number of leaves were not statistically significant and ranged between 7.2 leaves (T7) and 7.8 leaves (T3, T6); which were 2.9% and 11.4% higher than the 7 leaves in the control (Table 2).

In Experiment 2, it was observed that only the plants from the treatment with a dose of 175 g per ha^{-1} (T7) showed significantly greater heights than the control, with an average of 8.2 leaves, representing 28.1% more than the 6.4 leaves in the control (Table 2). The indicated dose of the herbicide may positively influence leaf formation per plant.

Martínez *et al.* (2021) observed that the number of leaves formed per plant decreases as the competition period between corn and weeds increases. This is an undesirable effect for plant productivity, as the number and size of leaves are determining factors for biomass production and grain yield in corn (Sánchez-Mendoza *et al.*, 2017). Based on this, it is important to consider that weed control in corn should be performed when the plant has between four and six leaves, because delaying control until the plant reaches ten leaves may lead to losses in production and economic income (Keller *et al.*, 2014).

Weight of corn plants (fresh and dry)

In this variable, the treatments had significant effects in both experiment 1 (Pr>F=0.0135*) and experiment 2 (Pr>F=0.0228*). In the first experiment, it was found that in the treatment with 160 g per ha^{-1} of Condraz® (T6), the plants had the highest averages with 69.6 g and 9.6 g for fresh and dry weight, respectively (Figures 1 and 2). However, these values were statistically different only from the control treatment T1 (35.6 g and 4.6 g for fresh and dry weight, respectively), exceeding it by 95.5% and 108.7% in weight (Figure 1).

In experiment 2, it was observed that in the treatments with Condraz® doses of 175 (T7) and 195 (T8) g per ha^{-1}, the plants reached the highest weights in both fresh (69.0 and 67.4 g) and dry (9.4 and 9.2 g) states. These were the two treatments that surpassed the control averages, which were 35.6 g and 4.0 g for fresh and dry weight. This indicates that, comparatively, the plants in T7 and T8 had weights that were 93.8% and 89.3% higher (fresh) and 135% and 130% higher (dry) than those in treatment T1 (Figure 1). The application of the highest doses of the herbicide was the most effective in significantly increasing the weight of the corn plants. These results differ from those reported by Tamayo *et al.* (2016), who determined that with applications of Condraz® at doses of 170 g per ha^{-1} in wheat cultivation, average weights ranged from 26.3 to 32.5 g for fresh plants and from 4.0 to 6.7 g for dry plants. However, the effects of the herbicide observed in the present study are similar to those of Vintimilla (2022), who evaluated the efficiency of herbicide mixtures for weed control in corn cultivation. Vintimilla noted that in the control treatment (without herbicide), the plants had lower weights due to the high incidence of weeds that exploited nutrients to the detriment of crop growth.

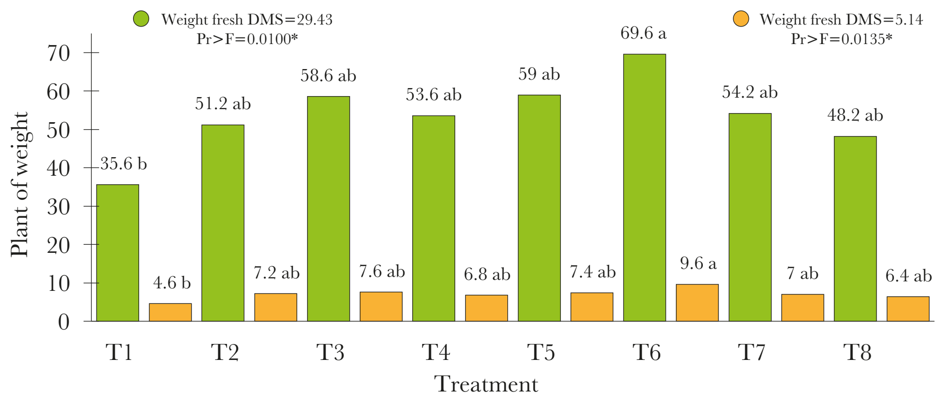


Figure 1. Effects of Condraz® (tritosulfuron+dicamba) doses on the fresh and dry weight of DK 357® corn plants at 36 days after planting (15 DAA) in Experiment 1. T1=Control (water), T2=Tritosulfuron+Dicamba (100 g ha^{-1}), T3=Tritosulfuron+Dicamba (115 g per ha^{-1}), T4=Tritosulfuron+Dicamba (130 g per ha^{-1}), T5=Tritosulfuron+Dicamba (145 g per ha^{-1}), T6=Tritosulfuron+Dicamba (160 g per ha^{-1}), T7=Tritosulfuron+Dicamba (175 g per ha^{-1}), T8=Tritosulfuron+Dicamba (195 g per ha^{-1}). DMS=Least Significant Difference of Tukey. Values with the same letters are not statistically different (Tukey, $\alpha \leq 0.05$).

Percentage of Leaf Cover of Corn Plants

The treatments caused highly significant differences in the evaluations conducted in Experiment 1, except in Evaluation 1 (Pr>Fc=0.1521NS), as well as in Experiment 2, except in Evaluation 1 (Pr>Fc=0.3916NS).

In Experiment 1, it was observed that the leaf cover behavior over time in Evaluations 2, 3, and 4 for the control treatment (no herbicide application) fit a linear regression model (R^2=0.96), with a 4.51% increase in leaf cover between each evaluation (Figure 2A). Similarly, in the treatments with Condraz® herbicide application, the model was similar (R^2=0.86), but with a 4.95% increase between evaluations (Figure 2B).

In Experiment 2, it was determined that the dynamics of leaf cover growth per plant in evaluations 2, 3, and 4 for the control treatment were represented by the linear regression model (R^2=0.94), with a 4.898% increase between evaluations (Figure 2C). In contrast, in the treatments with Condraz® herbicide application, the same model (R^2=0.76) indicated a 6.027% increase in cover between evaluations (Figure 2D). Based on these results, it can be stated that controlling dicotyledonous weeds with foliar application of Condraz® increased the leaf cover of corn plants by 9.7% to 23.1% compared to the control at 12 days after application (DDA). These results are similar to those reported by Callejas-Moreno *et al.* (2020), who found that in the control treatment (without herbicide application), weed cover was 72.5%, compared to an average of 25% in the herbicide-treated plots at 15 DDA.

Correlation Analysis

In Experiment 1, the response variables were highly significantly correlated (Pr>F=0.0001**). It was found that variables associated with plant growth, including height, collar diameter, number of leaves, and leaf cover, were positively and significantly associated with both fresh and dry plant weight. This is because as the plant grows, it tends

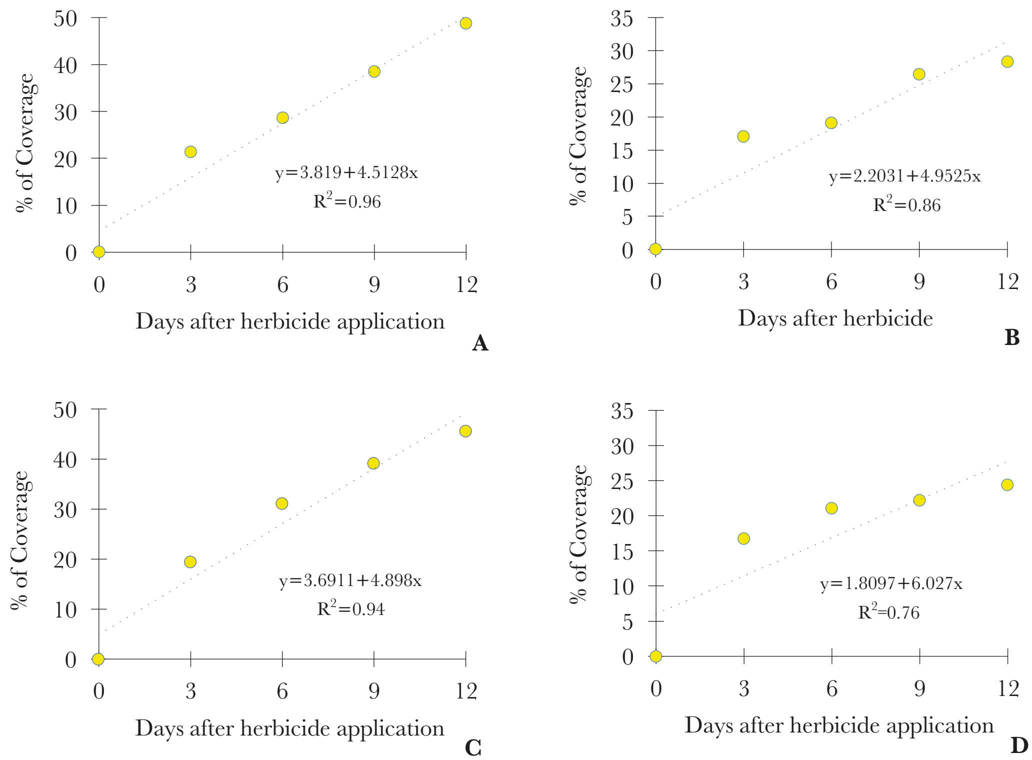


Figure 2. A. Linear regression model of leaf cover per corn plant in the control treatment in Experiment 1; B. Linear regression model of leaf cover per corn plant in the treatments with Condraz® herbicide application in Experiment 1; C. Linear regression model of leaf cover per corn plant in the control treatment in Experiment 2; D. Linear regression model of leaf cover per corn plant in the treatments with Condraz® herbicide application in Experiment 2.

to accumulate more biomass (Table 1). However, the correlation was negative between the percentage of phytotoxicity and both fresh plant weight and number of leaves, as herbicide-induced plant toxicity reduces plant development. There was also a negative and highly significant association between the number of live weeds and both height and fresh plant weight, as the presence of weeds negatively affects the increase in these two plant characteristics.

Table 3. Correlation analysis between response variables in Experiment 1.

	%E	% F	PF	PS	DT	A	NH
%F	0.42**						
PF	−0.41**	−0.36*					
PS	−0.40*	−0.35*	0.92**				
DT	-0.25^{NS}	−0.42**	0.72**	0.76**			
A	−0.47**	−0.45**	0.55**	0.58**	0.45**		
NH	-0.23^{NS}	−0.34*	0.65**	0.71**	0.69**	0.57**	
%CV	0.63**	0.26^{NS}	-0.29^{NS}	-0.30^{NS}	-0.08^{NS}	-0.26^{NS}	-0.14^{NS}

%E=Percentage of effectiveness. %P=Percentage of phytotoxicity. FW=Fresh weight of corn plants. DW=Dry weight of corn plants. SD=Stem diameter of corn plants. H=Height of corn plants. NL=Number of leaves per corn plant. %CV=Percentage of leaf coverage of the plant. NS=Not significant. *=Significant. **=Highly significant.

Table 4. Correlation Analysis between Response Variables of Experiment 2.

	%E	%F	PF	PS	DT	A	NH
%F	0.42**						
PF	−0.57**	-0.18^{NS}					
PS	−0.52**	-0.22^{NS}	0.90**				
DT	−0.60**	-0.24^{NS}	0.74**	0.68**			
A	−0.55**	0.33*	0.58**	0.60**	0.40**		
NH	−0.44**	-0.07^{NS}	0.74**	0.71**	0.61**	0.64**	
%CV4	0.77**	0.28^{NS}	−0.57**	−0.51**	−0.48**	−0.50**	−0.53**

%E=Percentage of effectiveness. %P=Percentage of phytotoxicity. FW=Fresh weight of corn plant. DW=Dry weight of corn plant. SD=Stem diameter of corn plant. H=Height of corn plant. NL=Number of leaves per corn plant. %CV=Percentage of foliar coverage of the plant. NS=Not significant. *=Significant. **=Highly significant.

In Experiment 2, the response variables were highly significantly correlated (Pr>F=0.0001). An interrelation of the variables similar to that observed in Experiment 1 was found; however, in this Experiment 2, the negative and highly significant correlation between the number of live weeds and the parameters, corn plant weight (fresh or dry), stem diameter, height, and number of leaves was more evident (Table 2). This is because these characteristics are negatively affected when weeds are growing in association with the economically important crop. These results are like those presented by Jamaica (2019), who reported that the crop exhibits better vegetative development in the absence of weeds.

CONCLUSIONS

The doses of Condraz® applied post-emergence did not show significant differences in biological effectiveness for eliminating dicotyledonous weeds growing in association with corn in the greenhouse. Low, medium, and high doses of Condraz® demonstrated 100% effectiveness in controlling dicotyledonous weeds. No dose of Condraz® caused phytotoxicity symptoms in corn plants. The control of dicotyledonous weeds with Condraz® increased the foliar coverage of corn plants by 9.7 to 23.1% compared to the control at 12 DAA. Growth variables correlated positively and significantly with the weight of corn plants. The incidence of dicotyledonous weeds correlated negatively with growth parameters and plant weight.

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Colegio de Postgraduados

Effects of Thinning on the Diversity, Composition, and Spatial Structure in a Mixed Temperate Forest

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ABSTRACT

Objective: To evaluate the effects of silvicultural thinning on tree diversity and stand structure in a temperate mixed forest.

Design/methodology/approach: Data were collected in a 1 ha research plot. Five scenarios were evaluated through computer simulations: no thinning (T1), thinning with removal of 25% of basal area (Gha^{-1}), (T2), thinning with removal of 25% of Gha^{-1} (T3), thinning with removal of 45% of Gha^{-1} (T4), and thinning with removal of 70% of Gha^{-1} (T5). The importance value index, alpha diversity, Pretzsch's A index and structural complexity index were estimated. A spatial distribution analysis was performed using the pair-correlation function $g(r)$.

Results: *Pinus douglasiana* and *Quercus resinosa* were the species of highest ecological value. Due to the removal effect, no significant changes in tree diversity were observed in the applied thinning scenarios. However, as thinning became more intense, at least one species (*Quercus candicans*) was lost. Thinning from below affected the oaks and thinning from above affected the pine species, which is also reflected in the spatial distribution of the remaining trees.

Limitations on study/implications: The analysis is static; therefore, it is recommended that a long-term study be conducted under varying ecological conditions.

Findings/conclusions: The effect of thinning on forest diversity, composition and structure depends on the type of thinning, condition of the structure, initial composition and intensity of removal. Thinning of less than 25% of the basal area, in the immediacy, allows timber harvesting without generating changes in the diversity, structure and composition of the temperate mixed forest under study.

Keywords: Simulations, pair-correlation function, structural complexity, silviculture, importance value index.

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INTRODUCTION

The configuration of forest canopy, shaped by natural succession and human intervention, constitutes a fundamental indicator of forest ecosystem functioning at different temporal and spatial scales (Gough *et al.*, 2022). Among silvicultural practices, thinning is an important and widely used activity in forest management (Franklin *et al.*, 2007). It involves the reduction of tree density through the selective removal of trees in relatively dense canopies (Liu *et al.*, 2019). This action redistributes available resources, improves nutrient availability, promotes the growth of remaining trees, and can create suitable conditions for species of commercial, ecological, or cultural importance (Smith *et al.*, 1996; Latterini *et al.*, 2023).

In addition to generating intermediate income from timber harvesting, thinning enhances pest control and fire prevention by promoting a more complex and diverse forest structure (Liu *et al.*, 2019; Latterini *et al.*, 2023). It also supports natural regeneration and emulates the effect of natural disturbances (Rubio-Camacho *et al.*, 2023), creating stand structures and spatial patterns that strengthen ecosystem resilience (Stephens *et al.*, 2008).

To study the effects of thinning, indices for characterizing the stand structure and species composition have been used (Gadow *et al.*, 2012; Prodan *et al.*, 1997). These indices comprise three main elements: 1) species composition, 2) dimensional diversity, and 3) spatial structure (Aguirre *et al.*, 2003; Gadow *et al.*, 2012; Pommerening, 2002). Utilizing these indices provides a detailed overview of the current state of forest stands and can be used to evaluate the effects of natural and anthropogenic disturbances on vegetation (Latterini *et al.*, 2023; Rubio-Camacho *et al.*, 2023). Furthermore, they are related to central ecosystem processes, including primary production, water use efficiency, and biogeochemical cycling rates (Gough *et al.*, 2022).

Through diversity indices, some studies in Mexico have demonstrated that silvicultural practices modify the stand structure and species diversity (Pérez-López *et al.*, 2020; Silva-González *et al.*, 2021; Soto Cervantes *et al.*, 2021). However, few studies assess the spatial structure of residual trees, and previous research is highly specific to certain species and regions. Therefore, it is necessary to expand knowledge to other species and forest areas in the country.

The use of experimental plots and the simulation of silvicultural practices in forest management offers multiple benefits, such as the validation and adjustment of management techniques before field application, providing crucial experimental control for the study of complex ecological interactions. These practices serve as essential platforms for decision-making by foresters. Additionally, thinning facilitates the anticipation and adaptation of forest management strategies to the effects of climate change, enables the testing of ecological restoration methods, and maximizes carbon sequestration.

The objective of this study is to analyze the immediate effects of thinning on forest composition and structure at the stand level. The research questions are: 1) How does thinning affect species diversity and composition? 2) What is the relationship between thinning types and structural complexity? and 3) Do different thinning methods generate heterogeneous spatial patterns? These questions are addressed through simulations of various thinning types with variable intensities. A mixed temperate forest dominated by *Pinus douglasiana* Martínez and *Quercus resinosa* Liebm., located in a protected natural area in the state of Jalisco, serves as a case study.

MATERIALS AND METHODS

Study Area

This study was conducted in the forests of the "Sierra de Quila" Flora and Fauna Protection Reserve, Jalisco, Mexico. The reserve is located in west-central Mexico at coordinates 20° 14.65' N to 20° 21.67' N and −103° 56.79' W to −104° 7.98' W. The area spans an altitudinal range from 1350 to 2550 meters above the sea level (INEGI, 2013). The vegetation is a mixed temperate forest, with representative species including *Pinus douglasiana* Martínez, *Pinus devoniana* Lindley, *Quercus resinosa* Liebm., and *Quercus obtusata* Bonpl (CONANP, 2000) (Figure 1).

Data Collection

The data were collected from a permanent research plot (100×100 m, 1 ha). Plot corners were delineated with 2 cm precision using a Ruide Total Station RTS-833 and georeferenced with a Topcon GR-5 Global Navigation Satellite System (GNSS). The plot was referenced to the Universal Transverse Mercator, Zone 13 North (UTM 13N) with central coordinates at 599,773.81 X and 2'245,771.62 Y (Figure 1). The plot was divided into 25 subplots using a Sokkisha TM10E theodolite (20×20 m, 0.40 ha), where trees with a diameter at breast height (d, cm) of 7.5 cm or greater were inventoried. The collected variables included: tree species, tree diameter (d, cm), total tree height (h, m), and crown diameter (dc, m). Additionally, using a total station, the spatial distribution of each tree (x and y coordinates) was obtained.

Simulation of Silvicultural Scenarios

The thinning scenarios evaluated were as follows: T1) no removal, T2) thinning from below with removal of 25% of total basal area (Gha^{-1}) ($d \leq 25$ cm), T3) thinning from above with removal of 25% of Gha^{-1} ($d \geq 30$ cm), T4) thinning from above with removal of 45% of Gha^{-1} ($d \geq 30$ cm), and T5) thinning from above with removal of 70% of Gha^{-1} ($d \geq 30$ cm). The cutting scenarios do not incorporate a temporal component, and only

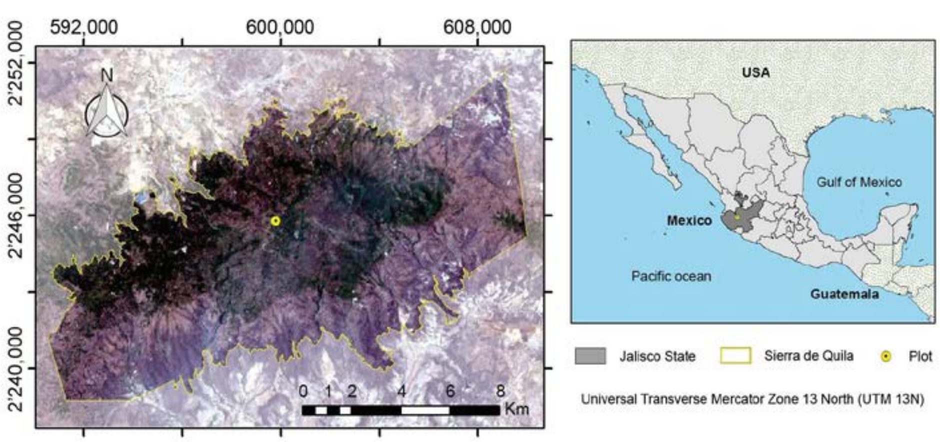


Figure 1. Location of the study area within the "Sierra de Quila" Flora and Fauna Protection Reserve, Jalisco, Mexico.

static data are analyzed. The criteria used for basal area removal in the iterations were: a) removal of individuals proportionally to basal area, with a maximum variation of 3%, b) residual tree distribution to protect soil conditions, and c) random elimination of trees.

Data Analysis

Before and after applying the iterations, the plot was characterized through stand structure indicators. To analyze the effects of thinning on tree diversity, composition, and structure, the following indices were considered: Species Importance Value Index (IVI), alpha diversity, Pretzsch's A index, and Enhanced Structural Complexity Index (ESCI). The IVI is an index used to rank the dominance of each species in mixed stands (Zarco-Espinosa *et al.*, 2010). The IVI per tree species was calculated by its abundance (number of individuals), dominance (based on crown cover area), and frequency (the number of plots were the species is present), and is presented in percentage values. Alpha diversity estimates species richness using the species richness index (S) and community structure through dominance by the Simpson's diversity index (λ) and evenness by the Shannon-Wiener index (H') (Moreno, 2001; Magurran & McGill, 2011). The A index (Pretzsch, 2009) evaluates the vertical distribution of species in a particular stand or forest. The ESCI allows a comparison of the surface area generated by connecting the tree-top of adjacent trees to form triangles with the total area covered by these projected triangles on a plane (Beckschäfer *et al.*, 2013) (Table 1).

To study the effects of thinning on spatial distribution, the pair correlation function $g(r)$ (Stoyan & Stoyan, 1994) was used. This function is the derivative of Ripley's K function (Ripley, 1977), and is described as: $g(r)=K'(r)/(2\pi r)$, where $K(r)$ is the average number of points within a circle of radius r from an arbitrary point, divided by the point pattern intensity (Stoyan & Stoyan, 1994; Wiegand & Moloney, 2014). The K function is:

$$K(r)=\frac{A}{n(n-1)}\sum_{i=1}^{n}\sum_{j\neq 1}^{n} l_{ij}(r)e_{ij}(r)$$

Where: A is the area, l_{ij} is the count function at the specific distance (r) from the reference point, and $e_{ij}(r)$ is the edge correction factor.

For all summary statistics used in this study, isotropic correction was applied (Ripley, 1977; Stoyan & Stoyan, 1994). When $g(r)=1$, it means that the points are randomly distributed at that distance. If $g(r)>1$, it indicates that the points are clustered at that distance, and if $g(r)<1$, it means that the points are regularly distributed. To address statistical significance ($\alpha=0.05$), significance bands were generated using Monte Carlo simulations based on 199 replications of a homogeneous Poisson process, which generate random data to serve as the null model. The bands were created using the fifth highest and the fifth lowest values from these simulations.

The analyses conducted in this study were performed using R 4.1.2 (R Core Team, 2021). Specific functions were created in this language for the development of thinning

Table 1. Species Diversity Indices. Where: S represents the number of tree species; p_i is the proportion of the i-th species; ln stands for natural logarithm; Z denotes the number of vertical zones and p_{ij} is the proportion of the i-th species in each j-th vertical zone, estimated by the equation $p_{ij} = n_{i,j}/N$, where $n_{i,j}$ is the number of records of the same species (i) in zone (j) and N=total number of recorded trees.

Index	Expression
Species richness (S)	Number of species
Simpson's diversity (λ)	$\lambda = \sum p_i^2$
Shannon's entropy (H')	$H' = -\sum_{i=1}^{S} p_i * \ln(p_i)$
Species vertical distribution (A)	$A = -\sum_{i=1}^{S} \sum_{j=1}^{Z} p_{ij} * \ln p_{ij}$

simulations, and custom codes were generated for the estimation of diversity and structural indices. For the spatial analysis, the SPATSTAT library was used (Baddeley *et al.*, 2015).

RESULTS AND DISCUSSION

The experimental plot contained 2 genera and 6 species: *Pinus douglasiana* Martínez, *P. lumholtzii* Rob. & Fern., *P. oocarpa* Shiede, *Quercus resinosa* Liebm., *Q. candicans* Née, and *Q. coccolobifolia* Trel. In the initial state of the stand, a density of 573 trees per hectare and a basal area of 27.8 m^2 per hectare were recorded. The most abundant species were *Quercus resinosa* and *Pinus douglasiana* (Table 2).

Table 2. Forest stand variables of thinning scenarios in a mixed temperate forest in Jalisco, Mexico. Where: Gha^{-1} is the basal area per hectare, Vha^{-1} is the volume per hectare, Nha^{-1} stands for the number of trees per hectare, $d_{1.3}$ is the diameter at breast height, h is the total height, and T_i is the iteration or evaluated scenarios.

Variable	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
	Pinus					***Quercus***				
Gha^{-1}	21.0	19.1	14.1	9.6	3.1	6.8	2.1	6.3	6.0	5.4
Vha^{-1}	284.5	268.8	184.5	118.7	27.7	69.4	26.7	62.6	58.6	50.1
Nha^{-1}	216.0	128.0	178.0	151.0	107.0	357.0	30.0	352.0	350.0	343.0
$d_{1.3}$ mean	31.2	41.8	27.9	24.9	17.9	14.2	28.7	13.9	13.7	13.3
$d_{1.3}$ sd	16.3	12.3	15.3	13.9	6.9	6.5	9.6	6.0	5.5	4.8
$d_{1.3}$ min	7.6	25.5	7.6	7.6	7.6	6.4	6.4	6.4	6.4	6.4
$d_{1.3}$ max	78.0	78.0	73.2	71.6	29.9	50.9	50.9	50.9	39.8	28.7
h mean	19.6	24.5	18.3	16.9	13.9	12.8	19.4	12.6	12.6	12.3
h sd	7.4	4.6	7.3	6.9	5.2	4.6	7.0	4.4	4.4	4.0
h min	4.5	14.7	4.5	4.5	4.5	4.2	5.2	4.2	4.2	4.2
h max	40.1	40.1	37.3	36.5	26.2	32.9	32.9	32.9	32.9	24.5
h dom	25.5	25.5	23.9	20.6	14.5	18.1	19.4	17.7	17.6	16.1

T1: No removal, T2: Thinning from below (T2), removal of 25% of basal area (Gha^{-1}), T3: Thinning from above (T3), removal of 25% of Gha^{-1}, T4: Thinning from above (T4), removal of 45% of Gha^{-1}, and T5: Thinning from above (T5), removal of 70% of Gha^{-1}.

The changes in the statistics of tree diameter ($d_{1.3}$) and height (h) before and after applying the different thinning scenarios showed a decrease in these metrics as the intensity of thinning increased. The basal area (Gha^{-1}) showed a similar pattern, fluctuating from 21 to 3.1 m^2 for *Pinus* and 6.8 to 2.1 m^2 for *Quercus*. The highest volume (Vha^{-1}) removed was for scenario T5 (276.1 m^3 ha^{-1}) and T4 (175.14 m^3 ha^{-1}). Scenario T2 had the least impact on Gha^{-1} (58.44 m^2 ha^{-1}) and Vha^{-1} (58.44 m^3 ha^{-1}), but it removed the highest number of trees per hectare. By targeting understory trees with $d \leq 25$ cm, T2 primarily affected young oaks, which are shade-tolerant and typically found in higher density below the main canopy. In contrast, scenario T5 primarily affected pine trees, leading to reductions in all stand indicators (Table 2).

P. douglasiana and *Q. resinosa* were identified as the species with the greatest ecological importance across the different thinning scenarios, with no significant changes observed after thinning. In contrast, *Q. candicans* and *P. oocarpa* had the lowest ecological values. Overall, there was an increase in the relative Importance Value Index (IVI) for the most represented species (Table 3).

Species richness prior to thinning was six species, which decreased to five in scenarios T3, T4, and T5 after thinning was simulated (Tables 3-4). In these scenarios, *Q. candicans* was the species that was removed. Despite this reduction in species richness, species diversity did not show apparent changes across the different thinning intensities. However, compared to T1, there was a decrease in the Shannon index (H') as the thinning intensity increased (Table 4).

The structural complexity of the forest stand decreased with increasing thinning intensity, indicating that the ESCI is particularly sensitive to silvicultural interventions. Scenario T2 showed the greatest impact on structural complexity, resulting in a 65% reduction in structural complexity. In contrast, T3 had the least impact, with only a 14% reduction, followed by T4 and T5 with 27% and 46%, respectively (Table 4).

Although silvicultural interventions are often used to regulate species composition and diversity in forest ecosystems (Latterini *et al.*, 2023), the thinning simulated in this study, did not lead to a strong decrease in species composition. Other research have documented that selective extractions can increase tree diversity and species richness over time, particularly when compared to more intensive methods (Torras & Saura, 2008). However, selective cuts may also result in the decline of old trees and negatively impact the establishment of shade-intolerant species (Jardel-Peláez, 2012).

Previous studies have demonstrated that thinning can increase structural complexity, as observed in *Pinus sylvestris* L. (Saarinen *et al.*, 2021). This contrasts with our study, where structural complexity of the stand decreased, which can be explained by the intensity and type of thinning used. Similar results have been reported in mixed pine-oak forests in Durango, Mexico, where low-intensity thinning did not significantly impact diversity and structure (Monárrez *et al.*, 2021; Delgado *et al.*, 2016).

Spatial Attributes

The results of the spatial analysis, including species and genera, illustrate the pattern of tree arrangement and are crucial for understanding forest ecosystems dynamics. Figure 2 shows the spatial distribution of the trees within the experimental plot.

Table 3. Ecological values of tree species by thinning scenario in a mixed temperate forest in Jalisco, Mexico. Where: Gha^{-1} is the Basal area per hectare (m^2), Nha^{-1} the Number of trees per hectare, RA is the Relative abundance (%), RD the Relative dominance (%), RF is the Relative frequency (%), and IVI the Importance value index (%).

Scenario	Species	Nha^{-1}	Gha^{-1}	RA	RD	RF	IVI
No removal (T1)	*P. douglasiana*	164	17	28.6	61.2	30.4	40.1
	Q. resinosa	309	5.4	53.9	19.4	30.4	34.6
	P. lumholtzii	45	3.6	7.9	13	20.3	13.7
	Q. coccolobifolia	47	1.2	8.2	4.3	15.2	9.2
	P. oocarpa	7	0.4	1.2	1.4	2.5	1.7
	Q. candicans	1	0.2	0.2	0.7	1.3	0.7
	Total	573	27.8	100	100	100	100
Thinning from below (T2) 25% removal of the Gha^{-1}	*P. douglasiana*	91	15.5	57.6	73	40	56.9
	Q. resinosa	34	3.3	21.5	15.6	23.3	20.1
	P. lumholtzii	20	1.3	12.7	6.1	23.3	14
	Q. coccolobifolia	9	0.7	5.7	3.1	8.3	5.7
	P. oocarpa	3	0.3	1.9	1.3	3.3	2.2
	Q. candicans	1	0.2	0.6	1	1.7	1.1
	Total	158	21.2	100	100	100	100
Thinning from above (T3) 25% removal of the Gha^{-1}	*Q. resinosa*	306	5.2	58.2	25.5	31.6	38.4
	P. douglasiana	132	11.2	25.1	55.2	31.6	37.3
	P. lumholtzii	38	2.8	7.2	13.8	18.4	13.1
	Q. coccolobifolia	44	0.8	8.4	4.2	15.8	9.4
	P. oocarpa	6	0.3	1.1	1.3	2.6	1.7
	Total	526	20.2	100	100	100	100
Thinning from above (T4) 45% removal of the Gha^{-1}	*Q. resinosa*	306	5.1	61	33.4	32.4	42.3
	P. douglasiana	113	6.7	22.5	43.7	31.1	32.4
	P. lumholtzii	31	2.1	6.2	13.9	17.6	12.5
	Q. coccolobifolia	46	1.1	9.2	7.3	16.2	10.9
	P. oocarpa	6	0.3	1.2	1.7	2.7	1.9
	Total	502	15.4	100	100	100	100
Thinning from above (T5) 70% removal of the Gha^{-1}	*Q. resinosa*	301	4.7	66.9	55.4	34.8	52.4
	P. douglasiana	82	2	18.2	24.3	30.4	24.3
	Q. coccolobifolia	42	0.7	9.3	8.2	15.9	11.1
	P. lumholtzii	21	0.9	4.7	10.9	15.9	10.5
	P. oocarpa	4	0.1	0.9	1.2	2.9	1.7
	Total	450	8.4	100	100	100	100

Table 4. Structure and diversity values of the residual tree stand by treatment. Where: Nha^{-1} is the number of trees per hectare and ESCI is the Enhanced Structural Complexity Index.

Scenario	Stand structure		Diversity			
			Richness	Dominance	Evenness	Structure
	Nha^{-1}	ESCI	Species Richness (S)	Simpson's diversity (λ)	Shannon's entropy (H')	A Pretzsch
T1	573	7.9	6	0.39	1.16	1.65
T2	158	2.8	6	0.40	1.18	1.82
T3	526	6.8	6	0.41	1.12	1.54
T4	502	5.8	5	0.44	1.07	1.65
T5	450	4.3	5	0.49	0.99	1.78

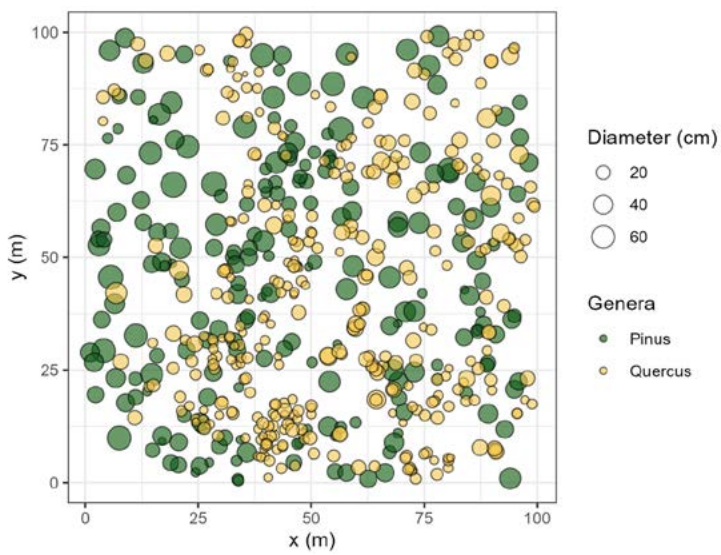
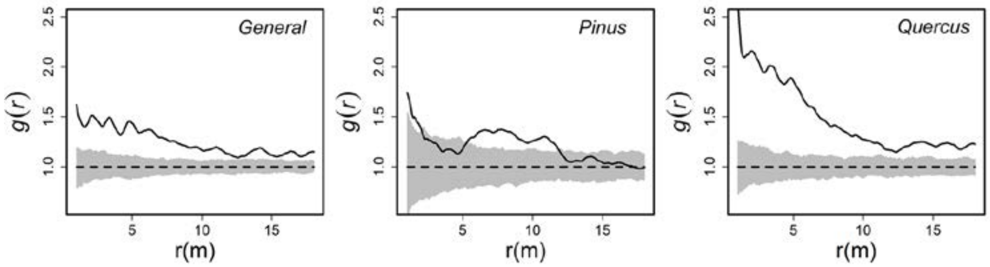


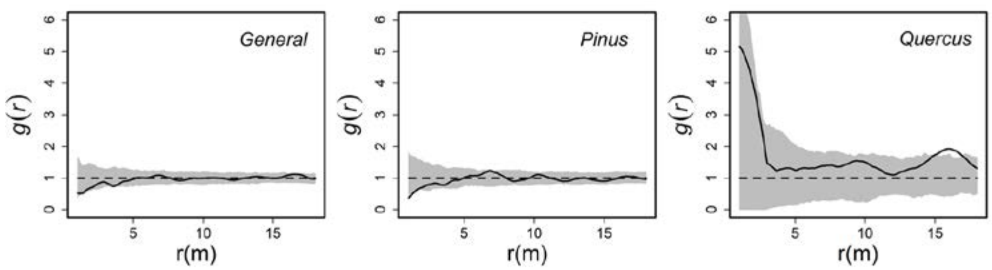
Figure 2. Spatial distribution of trees in the experimental plot. The circles represent the scale of the tree diameter and the colors indicate the genus.

The effects of thinning on spatial distribution varied according to the type and intensity of the intervention. Thinning from below (T2) significantly impacted the spatial distribution of oaks, leading to a random distribution at various scales (Figure 3b). In contrast, thinning from above had a more pronounced effect on the spatial distribution of pines (Figure 3c and Figure 3d). When analyzing all species collectively, thinning did not show significant effects. Scenario T1 resulted in a clustered distribution at different scales (Figure 3a), a pattern that was repeated in treatments T3-T5. However, T2 had a randomizing effect on the overall distribution, as the distribution of residual trees did not differ significantly from a random distribution or CSR (Figure 3b).

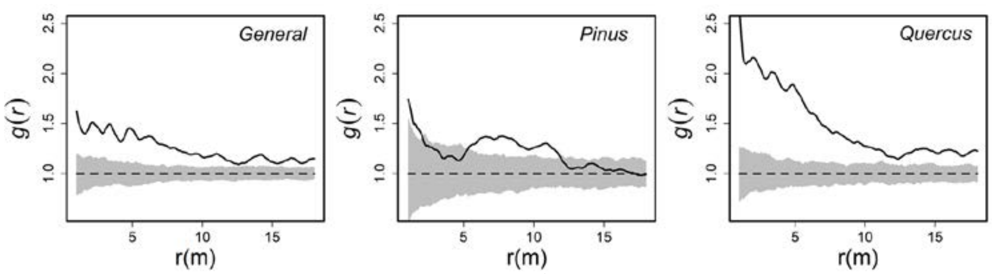
The impact of thinning on *Pinus* species was variable depending on the treatment applied. Specifically, T1 showed a clustered distribution between 4 and 9 meters, a trend that was repeated in treatment T3 and intensified in T4, increasing the clustering in the scale of 1 to 13 meters. Conversely, treatments T2 and T5 exhibited a randomizing effect, although T5 still displayed a slight clustering at a small scale of 7 to 9 meters (Figure 3e). In



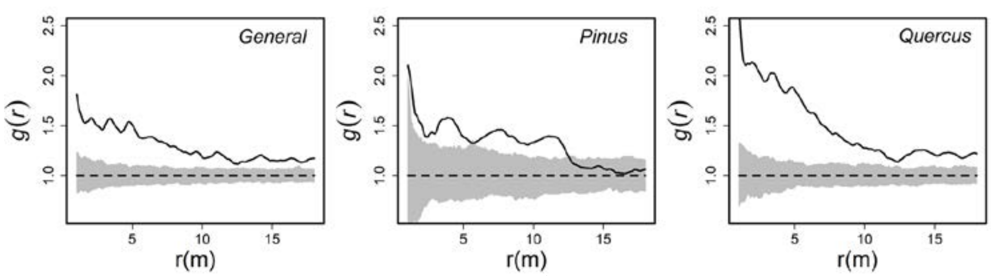
(**a**) T1 Original plot. No removal scenario.



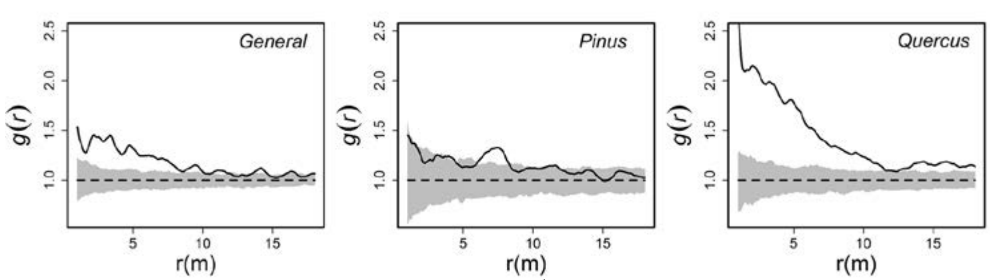
(**b**) T2 Thinning from below with removal of all trees with diameter <=25 cm.



(**c**) T3 Thinning from above, 25% removal of the Gha^{-1} (trees with diameter >=30 cm).



(**d**) T4 Thinning from above, 45% removal of the Gha^{-1} (trees with diameter >=30 cm).



(**e**) T5 Thinning from above, 70% removal of the Gha^{-1} (trees with diameter >=30 cm).

Figure 3. Spatial distribution by management scenario, where: $g(r)$ is the pair correlation function (with $g(r)=1$ indicating a random distribution, $g(r)>1$ a clustered distribution, and $g(r)<1$ indicating a regular distribution) and r denotes distance in meters.

the no-thinning scenario (T1), *Quercus* trees were clustered at all scales of analysis (0-18 m) (Figure 3a). This aggregated distribution was observed in most treatments, except for T2 (Figure 3b), where a random distribution was noted at most scales of analysis. This finding aligns with the observed effects of T2 on pines and on the overall species distribution.

In Mexico, studies have documented that forests undergoing silvicultural interventions often exhibit random distribution patterns (Corral *et al.*, 2005). Similar findings were observed in our study; for instance, T2 influenced the spatial structure of *Quercus* and *Pinus*, resulting in a random distribution of residual trees. Graciano *et al.* (2020), found that trees in five *Pinus durangensis* forest associations displayed a random distribution and high heterogeneity, which were attributed to the species composition and the management practices applied.

CONCLUSIONS

In this study, silvicultural treatments were simulated to evaluate their impact on the structure and composition of species in a temperate forest. It was concluded that the effect of thinning on the residual stand's structure depends on factors such as the type of thinning (thinning from above or from below), the initial condition of the stand, its composition, and the intensity of removal. To optimize outcomes and develop management prescriptions that balance wood production with the conservation of diversity, composition, and stand structure, it is essential to consider the ecological conditions of the study areas as well as the types of removal scenarios or silvicultural practices. Implementing silvicultural simulations in experimental research plots supports the sustainable management of forests, by allowing for the emulation of both natural and anthropogenic disturbances, which is crucial for effective forest management.

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Colegio de Postgraduados

A mixed-effects height-diameter model for *Pinus douglasiana* Martínez in temperate forests of Jalisco, Mexico

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ABSTRACT

Objective: This study aims to develop a generalized mixed-effects model for predicting tree-height of *Pinus douglasiana* in the natural forests of Jalisco, Mexico.

Design/methodology/approach: For this study, we utilized 2,921 pairs of tree-height measurements collected from 65 permanent plots (each 50×50 m) in the study area. For each plot, we estimated the tree density as the number of trees per hectare (N, ha^{-1}), the basal area per hectare (G, m^2 ha^{-1}) and the quadratic mean diameter (dg, cm). Subsequently, we tested several models from the literature and developed a generalized mixed-effects model for tree height prediction.

Results: The Gompertz base model outperformed the other local models, achieving an R^2 of 0.75 and an RMSE of 3.13. Including the stand variables (N, G, and dg) and incorporating random effects significantly improved the model fit, resulting in an R^2 of 0.89 and an RMSE of 2.09 m. Calibration and validation steps revealed that selecting the three thickest trees is effective for estimating random effects in new plots or stands, with the RMSE and R^2 being 2.59 and 0.83, respectively.

Limitations on study/implications: The present model can be applied in areas where *P. douglasiana* is naturally distributed. However, it is advisable to calibrate the model using a sub-sample to achieve more accurate predictions of tree heights in new plots or areas.

Findings/conclusions: The Gompertz function was used as the base function to develop the final generalized mixed-effects model for predicting the height of *Pinus douglasiana*. The inclusion of stand variables (N, G, and dg) along with random effects improved the model fit. This model represents a new and more accurate tool for predicting the height of *P. douglasiana* in areas where it is naturally distributed.

Keywords: Calibration, Cross-validation, generalized model, natural forests, forest inventory.

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INTRODUCTION

To foster sustainable forest management, it is crucial to employ tools that offer precise insights into the growth and development of forests and stands. For instance, incorporating both total tree-height (h) and diameter (d) into models enables accurate prediction of individual tree and stand-level volume. This approach also serves as an indicator for evaluating site productivity through estimation of dominant tree-height (López *et al.*, 2003). However, measuring total tree-height poses practical challenges, leading to increased estimation errors, time consumption, and costs during inventories. Nonetheless, employing tools such as allometric functions that model the height-diameter relationship (h-d) offers a method to estimate tree-height indirectly, thereby optimizing sampling efficiency and reducing inventory costs (Calama & Montero, 2004; Mehtätalo *et al.*, 2015).

Various functions, encompassing both linear and nonlinear forms, have been employed to develop height-diameter models. A comprehensive compilation can be found in Huang *et al.* (1992), Guzmán *et al.* (2019), and Ogana (2020). Functions that solely account for diameter are referred to as local models; however, these may not universally apply across different forest growth conditions or silvicultural treatments, as total tree-height can vary with site quality or stand density (Prodan *et al.*, 1997; Corral *et al.*, 2019; Guerra-De la Cruz *et al.*, 2019). Hence, an alternative approach involves generalized models that incorporate stand-level variables as predictors (Corral *et al.*, 2019; Rubio-Camacho *et al.*, 2022).

Generalized equations for height-diameter relationships are often fitted using the mixed-effects modeling (MEM) approach. Compared to ordinary least squares (OLS), MEMs offer greater flexibility and accuracy considering the hierarchical structure typical of forest inventories (López *et al.*, 2012). Unlike OLS models, MEMs overcome the independence assumption among observations, resulting in improved tree-height estimation (Castedo *et al.*, 2006). Moreover, MEMs can be calibrated using height measurements from a small subsample of trees. This calibration allows for the estimation of random parameters specific to a particular plot or stand, differing from those used in the model fitting process, thus enhancing their practical utility in forest management (Corral *et al.*, 2019; Rubio-Camacho *et al.*, 2022).

In the Mexican forestry sector, mixed-effects models have been sparingly utilized, primarily developed for northern (Vargas *et al.*, 2009; Corral *et al.*, 2019) and central regions (Rubio-Camacho *et al.*, 2022). However, in western Mexico, most studies do not include random effects. Therefore, there is a need to develop such tools for economically and ecologically important species in this region.

The purpose of this study was to develop a generalized model for tree-height prediction of *Pinus douglasiana* Martinez in the temperate forests of Jalisco, Mexico. The specific objectives were: i) to evaluate the effectiveness of commonly used local equations in forestry, ii) to identify and select stand variables as predictors, iii) to fit a generalized mixed-effects model, and iv) to calibrate and validate the developed mixed-effects model. The proposed model aims to provide an efficient tool applicable in western Mexico and within the natural distribution areas of the species.

MATERIALS AND METHODS

Study area

This research was conducted in the Sierra del Sur and Sierra Occidente regions of Jalisco, focusing on areas where *Pinus douglasiana* is naturally distributed. The species exhibits a broad altitudinal range from 1,100 to 2,500 meters above sea level, with optimal growth observed between 1,700 and 2,400 meters above sea level (PRODEFO, 2011). It thrives in temperatures ranging from 17 to 23 °C and typically experiences rainfall between 700 and 1600 mm annually, peaking in August and September. *Pinus douglasiana* prefers rich, well-drained soils of moderate depth (PRODEFO, 2011), and is commonly found in association with genera such as *Pinus*, *Quercus*, *Ostrya*, *Carpinus*, *Juglans*, and *Abies*, which are among the most significant (Sandoval, 2010).

Database

A total of 65 sampling plots of 50×50 m were selected where *P. douglasiana* was present (Figure 1). For each tree with a normal diameter equal to or larger than 7.5 cm, information was collected on species, tree-diameter measured at 1.3 m (d, cm) with a Jackson MS diameter tape, total tree-height (h, m) with a Suunto clinometer, among others. The database was generated by the Comisión Nacional Forestal (CONAFOR) under the project "Monitoreo Nacional Forestal: Red de Sitios Permanentes de Investigación Forestal y de Suelos" (CONAFOR, 2024). Using the collected data from each plot, the following stand variables were estimated: number of trees per hectare (N, ha^{-1}), basal area per hectare (G, ha^{-1}), quadratic mean diameter (dg, cm), dominant tree-height (H_D, cm) estimated from the 100 thickest trees per hectare. A total of 2,921 trees were measured, and a detailed description of the database is provided in Table 1 and Figure 2a.

Base model selection

The height-diameter (h-d) relationship was characterized using four commonly used local equations, which utilize diameter as the independent variable. These equations, widely applied in both national (Corral *et al.*, 2019; Rubio-Camacho *et al.*, 2022) and international (Mehtätalo *et al.*, 2015) studies, were evaluated and compared (Table 2). Each equation was fitted to the dataset using nonlinear least squares (NLS) regression.

The goodness of fit was assessed using metrics such as root mean square error (RMSE, 5), Akaike information criterion (AIC, 7), and Bayesian information criterion (BIC, 8).

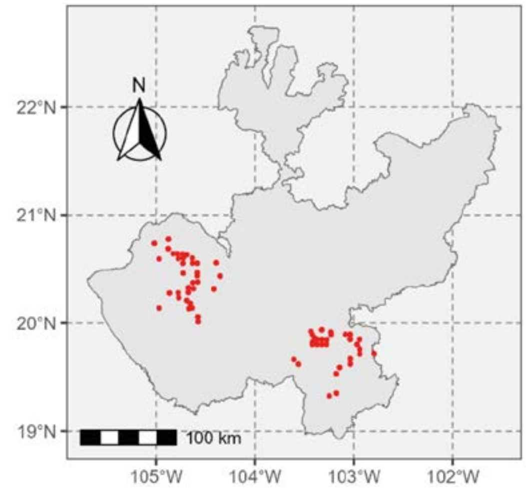

Figure 1. Location of the study area in the state of Jalisco, with sampling sites indicated in red.

Table 1. Descriptive statistics of the database.

Variables	Mean	Min.	Max.	Std.
d, cm	19.36	12.31	48.76	6.82
h, m	12.94	8.19	26.34	4.09
dg, cm	21.37	12.89	51.33	7.58
H_D	17.68	10.63	29.51	5.62
G, ha^{-1}	25.64	5.27	46.56	8.60
N, ha^{-1}	952.28	64.00	2276.00	638.48

Where: d=tree-diameter; h=total tree-height, dg=quadratic mean diameter; H_D=dominant tree-height; G=basal area per hectare; N=number of trees per hectare.

Table 2. List of equations tested for selecting the base model.

Model name	Expression	Eq.
Curtis (1967)	$h=1.3+\frac{\beta_0 \cdot d}{(1+d)^{\beta_1}}$	1
Schumacher (1939)	$h=1.3+\beta_0 \cdot \exp\left(-\beta_1 \cdot d^{-1}\right)$	2
Näslund (1936)	$h=1.3+\frac{d^2}{\left(\beta_0+\beta_1 \cdot d\right)^2}$	3
Gompertz (1825)	$h=1.3+\beta_0 \cdot \exp\left(-\beta_1 \cdot \exp\left(-\beta_2 \cdot d\right)\right)$	4

Where: h=total tree-height (m); d=tree-diameter (cm); β_i=model parameters to be estimated.

Additionally, the adjusted coefficient of determination R^2 (6) was used to measure the proportion of variance explained by each equation. The model fitting was performed using the 'nls' function in R v 4.3.2 (R Core Team, 2023).

The expressions of the goodness-of-fit statistics are as follows:

$$RMSE=\sqrt{\frac{\sum_{i=1}^{n}\left(y_i-\hat{y}_i\right)^2}{n-p}} \quad (5)$$

$$R^2=1-\frac{(n-1)\sum_{i=1}^{n}\left(y_i-\hat{y}_i\right)^2}{(n-p)\sum_{i=1}^{n}\left(y_i-\bar{y}_i\right)^2} \quad (6)$$

$$AIC=n\ln\left(\frac{SSR}{n}\right)+2p \quad (7)$$

$$BIC = n\ln\left(\frac{SSR}{n}\right) + p\ln(n) \qquad (8)$$

Where: $y_i, \hat{y}_i, \bar{y}_i$ =observed, predicted and mean values, respectively, n=total number of observations, p=number of model parameters, SSR=sum of the squares of the residuals, ln=natural logarithm.

Generalized mixed-effects model

Once the most accurate local model was chosen, it was expanded by incorporating stand variables as predictors. Various combinations were tested to significantly improve the precision of total height estimation. Similar studies recommend selecting stand variables highly correlated with the parameters of the chosen local equation (Bronisz & Mehtätalo, 2020; Rubio-Camacho *et al.*, 2022; Teshome *et al.*, 2024). Finally, the resultant generalized equation was utilized to develop a mixed-effects model following the methodology proposed by Pinheiro & Bates (2013).

It is important to note that both tree-height and tree-diameter are variables typically measured within sampling plots, which are located in stands across different geographic zones. This nested structure (trees nested within plots) results in a lack of independence among observations, as data from the same sampling unit (plot) tend to be more similar (Fox *et al.*, 2001). Various methodologies have been developed to address this issue, with mixed-effects modeling approximation being one of the most widely adopted (Gregoire *et al.*, 1987; Calama and Montero, 2004). This approach simultaneously estimates fixed parameters, which are consistent across the population, and random effects specific to each plot, ensuring robust parameter estimation and associated standard errors. For a comprehensive understanding of mixed-effects models in this context, refer to Corral-Rivas *et al.* (2019), Mehtätalo *et al.* (2015), and Rubio-Camacho *et al.* (2022).

In this study, the nonlinear generalized model was linearized using the first-order Taylor series expansion and fitted using the restricted maximum likelihood procedure. Model fitting was carried out using the 'nlme' package in the R statistical software (R Core Team, 2023), employing the estimated best linear unbiased predictor (EBLUP), a method developed by Pinheiro and Bates (2000).

Mixed-effects model calibration and validation

Calibration involves estimating site-specific random effects using data independent of the model fitted. Following the approach recommended by Yang and Huang (2013), this study employed the first-order conditional expectation (FOCE) approximation for estimating random parameters. This method aligns with the iterative process used in parameter fitting to obtain the value of $\hat{b}$, as described by Lindstrom and Bates (1990).

$$\hat{b}_i = \hat{D}Z_i^T\left(\hat{R}_i + Z_i\hat{D}Z_i^T\right)^{-1}\left[y_i - f\left(x_i, \hat{\beta}, \hat{b}\right) + Z_i\hat{b}\right]$$

Where: $\hat{b}_i$ =random effect, f =non-linear function, in this case the tree h-d generalized model, $\hat{\beta}$ =fixed-effects parameter vector, i=sampling plot, $\hat{D}$ =variance-covariance matrix $(q*q)$ linked to the random effect, $\widehat{R}_i$ =error term variance-covarience matrix $(m*m)$, Z_i=a matrix $m*q$ of the partial derivatives of the random effects evaluated at $\hat{b}=0$.

To calibrate the random effects for a new plot, it is necessary to sample tree heights from that plot. Various sampling alternatives have been proposed in previous studies (Corral *et al.*, 2019; Ogana *et al.*, 2020). In this research, a sample comprising the three tallest trees was selected, in line with recommendations from similar studies (Rubio-Camacho *et al.*, 2022; Teshome *et al.*, 2024). The calibration process was integrated with cross-validation techniques to estimate tree-level heights in plots not utilized in the parameter fitting process (Hastie *et al.*, 2009). Specifically, one 'plot' was omitted in each iteration to validate the fixed and calibrate the random effects simultaneously, following the approach outlined by Rubio-Camacho *et al.* (2022). This process was tested using the goodness-of-fit statistics (RMSE and R^2) to evaluate the final mixed-effects generalized model.

RESULTS AND DISCUSSION

Local models

In the first step, all four local functions were fitted using ordinary nonlinear least squares (NLS), revealing that each parameter was statistically significant at the 5% level. These functions exhibited robust fitting capabilities, explaining approximately 75% of the total height variability, with root mean square error (RMSE) values ranging from 3.1 to 3.2 meters. The Akaike information criterion (AIC) and Bayesian information criterion (BIC) showed minimal differences across the functions (Table 3). Given the parity in goodness-of-fit statistics, further assessment involved graphical analysis of bias, AIC, and RMSE to evaluate predictive performance across diameter classes. Notably, the Gompertz function (Figure 2b) demonstrated superior performance with lower associated errors and consistent bias behavior across different diameter classes. Based on these outcomes, the Gompertz (1825) function emerges as the most appropriate model for characterizing the total height of Pinus douglasiana in Jalisco.

Generalized mixed-effects model

During the addition and combination of stand variables into the local function of Gompertz (1825), challenges with fit and convergence were encountered. The inclusion of dg, N, and G resulted in significant improvements in precision. Dominant tree-height

Table 3. Values of goodness-of-fit statistics for the local equations fitted to the height-diameter data of *P. douglasiana*.

Equation	RMSE	R^2	AIC	BIC
1	3.2109	0.74	15196.4	15214.3
2	3.2255	0.74	15223.2	15241.1
3	3.1445	0.75	15073.7	15091.6
4	3.1380	0.75	15062.4	15086.4

Where: RMSE=Root mean squared error; R^2=coefficient of determination; AIC=Akaike's information criterion; BIC=Bayesian information criterion.

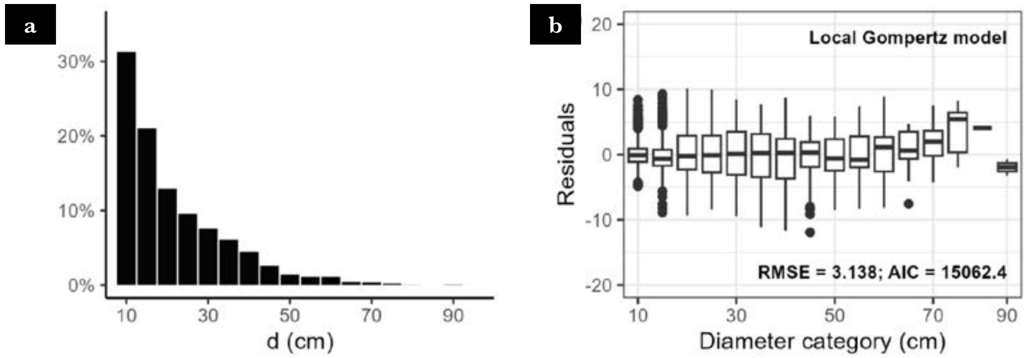

Figure 2. illustrates: a) the diameter distribution of the *Pinus douglasiana* database, and b) the residuals categorized by diameter for the local model using the Gompertz function.

(H) was deliberately omitted due to its potential for increased field sampling efforts, aligning with the practices of Bronisz & Mehtätalo (2020) and Rubio-Camacho *et al.* (2022). Ultimately, the generalized function explained approximately 80% of the variance in height with a relatively low root mean square error (RMSE), as summarized in Table 4. This finding is consistent with the results reported by Ogana (2019) for forests in Nigeria. The final expression of the generalized height-diameter function, fitted using ordinary nonlinear least squares (ONLS), is as follows:

$$h = 1.3 + \left(\beta_0 + \beta_3 \cdot \log\left(d_g\right)\right)\exp\left(-\left(\beta_1 + \beta_4 \cdot \left(\frac{G}{N}\right)\right) \cdot \exp\left(-\beta_2 \cdot d\right)\right) \quad (9)$$

Where: h=tree height; d=tree diameter; $\beta_0 \ldots \beta_4$=fixed effects, d_g=quadratic mean diameter; G=basal area per hectare; N=number of trees per hectare.

To formulate the mixed-effects model, we initially fitted the generalized equation using all possible combinations of random effects. However, some combinations failed to converge. The combination incorporating random parameters linked to β_0 and β_1 yielded the lowest AIC and BIC values, aligning with the recommendation by Castedo-Dorado *et al.* (2006) for selecting the mixed-effects model based on these criteria. This outcome indicates that the parameters governing the asymptote and shape of the height-diameter curve vary among sites, and this variability is effectively accounted for by the model in conjunction with stand variables. The expression of the mixed-effects model is detailed in equation 10, and corresponding goodness-of-fit statistics and estimators are presented in Table 4.

$$h = 1.3 + \left(\beta_0 + u_{0i} + \beta_3 \cdot \log\left(d_g\right)\right)\exp\left(-\left(\beta_1 + u_{1i} + \beta_4 \cdot \left(\frac{G}{N}\right)\right) \cdot \exp\left(-\beta_2 \cdot d\right)\right) \quad (10)$$

Where: h=tree height; d=tree diameter; $\beta_0 \ldots \beta_4$=fixed effects; u_{0i} y u_{1i}=random effects on β_0 y β_1; d_g=quadratic mean diameter; G=basal area per hectare; N=number of trees per hectare.

Table 4. Parameter values and goodness-of-fit statistics of the generalized equations for *Pinus douglasiana*.

Components	Base model (NLS)	Mixed model
Fixed effects		
β_0	−5.70 (1.950)	−2.76 (6.053)
β_1	1.84 (0.075)	1.57 (0.164)
β_2	0.08 (0.003)	0.07 (0.002)
β_3	9.01 (0.554)	7.7 (1.850)
β_4	−8.67 (2.175)	−9.89 (2.872)
Random effects variance		
sd(u_{0i})	-	4.11
sd(u_{1i})	-	0.52
Cor(u_{0i}, u_{1i})	-	0.37
Model performance		
RMSE	2.98	2.09
R^2	0.78	0.89
AIC	14767.06	12678.45
BIC	14802.97	12714.37
Cross-Calibration-Validation (mixed-effects model)		
Sub-sample trees (n)	-	3
RMSE	-	2.59
R^2	-	0.83

Where: β_0... β_4=fixed parameters; sd(u_{0i}) y sd(u_{1i})=Standard deviation of the random effects; Cor=Random effects correlation; RMSE=Root mean squared error; R^2=coefficient of determination; AIC=Akaike's information criterion; BIC=Bayesian information criterion.

The development of the mixed model demonstrates an 11.25% increase in explained variability compared to the base generalized model, confirming significant variability among plots (Table 4). Examination of residual plots for the mixed model (Figure 3) indicates consistent variance homogeneity across the entire range of predicted values.

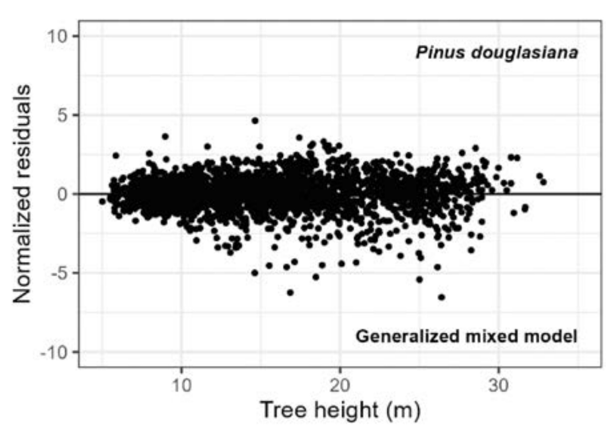

Figure 3. Distribution of residuals from the final model.

This, coupled with our use of cross-sectional data where observations within the same plot are uncorrelated, suggests that inclusion of a "weight factor" in the error variance of the variance-covariance matrix for each study plot is unnecessary (Fang & Bailey, 2001; Corral *et al.*, 2019).

Model calibration and validation

In this study, calibration and validation of the mixed-effects model were conducted simultaneously. To optimize sampling efforts and reduce associated inventory costs, the height of the three thickest trees in each plot or sampling site was measured (Table 4). To assess the improvement in goodness-of-fit statistics with the selected calibration approach, the accuracy achieved relative to the fit of the generalized model by ordinary nonlinear least squares (NLS) was evaluated. The RMSE value showed marginal reduction, and absence of collinearity in residuals supports the recommendation of using the generalized mixed model with random parameter estimation based on a subsample of three trees.

Several studies have compared different tree combinations for calibration (Bronisz & Mehtätalo, 2020; Ogana *et al.*, 2020; Teshome *et al.*, 2024). However, in line with the recommendation by Rubio-Camacho *et al.* (2022), the generalized mixed model offers significant practical advantages for field applications, requiring measurement of only three subsample trees.

CONCLUSIONS

The Gompertz model emerged as the most effective predictor of the tree-height for *P. douglasiana* trees among the local models assessed. Incorporating stand variables (N, G, and dg) notably enhanced height predictions. Furthermore, adding the random effects successfully captured plot-to-plot variation, resulting in improved height estimations.

Regarding calibration, measuring the heights of the three thickest trees proved to be a robust sampling method for estimating random effects, thereby enhancing height predictions for trees in new plots or stands. However, in cases where this subsample is unavailable, the mixed-effects model remains valuable by setting random effects to zero. However, for optimal accuracy, the calibration technique is recommended when applied to new plots or sites. The model developed in this study is expected to assist foresters in reducing inventory costs and accurately estimating missing or erroneous height data within inventory databases. Ultimately, this model provides a reliable tool for height estimation in natural forests of *P. douglasiana* in Jalisco state and other regions where the species is naturally distributed.

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and valuable contributions that have benefitted the people of Mexico, marking a significant milestone over decades. This manuscript stands as our tribute, commemorating 50 years of their remarkable achievements.

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Colegio de Postgraduados

Salinity and microbiological risk assessment in surface runoff and main tributaries to La Vega Dam in Teuchitlán, Jalisco, Mexico

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ABSTRACT

Objective: This research aimed to evaluate the water quality of the main tributaries to the La Vega Dam for irrigation purposes through the physicochemical and microbiological parameters.

Design/methodology/approach: The 10 main tributaries to the La Vega Dam were selected. The variables evaluated were pH, Temperature, Electrical Conductivity, Total Dissolved Solids and Dissolved Oxygen, boron calcium, magnesium, sodium, SAR, and RSC.

Results: 50% of samples presented low sodicity risk, 10% medium risk, 20% and 20% very high risk. In the RSC results, only one site (10%) presented a low risk, and the remaining four tributaries (40%) of this area presented a medium risk. Five sites (50%) located east of the dam presented a high risk. Also, high boron concentration was observed, corresponding to El Salado River. Fecal pollution was detected in 100% of sites, limiting their use as irrigation water.

Limitations on study/implications: Continuous water quality monitoring of the main tributaries to and into La Vega Dam should be followed up.

Findings/conclusions: The most significant contribution of contamination to the dam was observed in the eastern part, with the entrance of El Salado River. High concentrations of boron were also observed. A high concentration of total and fecal coliforms was also found, in addition to the presence of *Pseudomonas* spp., which indicates fecal contamination. The effluent at the east of La Vega Dam is unsuitable for irrigation.

Keywords: biological hazard, dam, monitoring, sustainability, water bodies.

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INTRODUCTION

La Vega Dam is the largest water body in the Valles Region, located in the Central West of Jalisco State, in the municipality of Teuchitlán. Its main tributaries are the El Salado River, Teuchitlán River, Arroyo Chapulimita (stream), and Arroyo Grande (stream). El Rincón springs and four springs are north of the dam, converging the reservoir as a single

tributary (CEA, 2011). Additionally, surface runoff typically originates during the rainy season and can contribute to chemical and biological contamination in the reservoir (Czemiel, 2010; Vialle *et al.*, 2011). La Vega Dam is of socio-economic importance in the region as it captures and distributes water for the irrigation district users of Ameca, Jalisco (CEA, 2011). The main activity in the communities surrounding the reservoir is agriculture, especially the cultivation of sugar cane, corn, and wheat.

On the other hand, water quality standards provide the basis for controlling polluting activities and guide identifying and developing quality criteria (Malakar *et al.*, 2019; Qadir *et al.*, 2021). This endures that water use and management of its watershed can be appropriately designated (Heiskary & Wilson, 2005; Park *et al.*, 2018). In the case of water for irrigation use, the content and type of salts are crucial to determine its quality; depending on these factors, irrigation water can favor or correct alkaline or saline soils (Gurjinder *et al.*, 2017; Amer, 2010). A high concentration of salts present in irrigation water reduces water availability for crops. Thus, the plant will have to work harder to absorb water, even suffering physiological stress due to dehydration, which affects its growth (Safdar *et al.*, 2019; Kiremit & Arslan, 2016). Determining the type of salts in irrigation water is essential since they also alter the soil and modify its structure, reducing infiltration (Cuellar *et al.*, 2015; Can *et al.*, 2008). The parameters that must be evaluated to determine the water quality for irrigation use are the following: electrical conductivity, pH, total amount of dissolved salts; calcium, magnesium, sodium, potassium, nitrates, carbonates, bicarbonates, chlorides, boron, the Sodium Absorption Ratio (SAR) and Residual Sodium Carbonate (RSC) levels (Marín *et al.*, 2002). Sodicity causes impairment of water mobility in different soil layers, and as a result the growing plant's roots may not have adequate water.

The most common method for measuring water and soil sodicity is calculating the sodium absorption ratio (SAR). The SAR defines sodicity in terms of the relative concentration of sodium (Na) compared to the sum of calcium (Ca) and magnesium (Mg) ions. The SAR assesses the potential infiltration problems due to sodium imbalance in water. For the SAR calculation, the Na, Ca, and Mg ions concentration is estimated in milli-equivalents per liter (Meq L^{-1}) (Tanji, 1990). According to the international criteria established for irrigation water (Ayers & Westcot, 1994), a scale determines the sodium risk in water.

Conversely, considering the broad exposure to different factors that can alter the water quality of the tributaries to a water body, the vital liquid can become a transmitter of various diseases (Tarqui *et al.*, 2016). In this way, microbiological analysis is another critical examination for determining water quality. Total Coliform bacteria are a collection of microorganisms that live in large numbers in the intestines of humans and warm-blooded animals. A specific subgroup of this collection is the fecal Coliform bacteria (Erdal *et al.*, 2003). The Ecological Criteria for Water Quality CE-CCA-001/89 mentions maximum permissible limits (MPL) for fecal Coliforms of 1000 with the Most Probable Number (MPN/100 ml) technique in the case of irrigation water. According to the literature, fecal coliforms in aquatic environments indicate that the water has been contaminated with fecal matter from humans or animals. Consequently, the water source is contaminated by pathogens, bacteria, or viruses that cause diseases that can also exist in fecal matter (De

La Mora *et al.*, 2013). The objective of this research was to evaluate the quality of water for use in irrigation in the main tributaries of La Vega Dam through physicochemical and microbiological parameters.

MATERIALS AND METHODS

Area of study location

Within the hydrological regions in the Jalisco State, there is the Ameca region, within which La Vega-Cocula Dam, the Ameca-Atenguillo River, and the Ameca-Ixtapa River are located. La Vega Dam is in the municipality of Teuchitlán, and its reservoir is 9.5 km south of Teuchitlán town. The reservoir has several tributaries: from the north, it receives the contribution of the Teuchitlán River, which is permanent. However, it is also a reservoir for the municipal discharges of Teuchitlán that eventually flow into the reservoir. In the north, a series of El Rincón springs flow into the reservoir in addition to the Teuchitlán River. In the west, La Vega Dam is fed by the contributions of the Grande or La Mora streams and the Chapulimita stream. However, the most significant contribution is in the southeast, corresponding to El Salado River, the reservoir's most important permanent recharge (CEA, 2011).

Selection of sampling sites

The 10 main tributaries to La Vega Dam were selected; the coordinates of the sites are shown in Table 1 and Figure 1. A land vehicle was used to collect samples, and the sites were located using a GPS (Etrex Garmin® brand).

Collection and transfer of samples

The collection of water samples was carried out following the technical guidelines established in the Mexican Official Norm NOM-AA-14-1980 "Receiving bodies, sampling," published in the Federation Official Gazette on Friday, August 27, 1980. The sampling was carried out by trained personnel from the National Institute of Forestry,

Table 1. Selection of sampling site coordinates.

Sampling site		Geographic coordinates	
Number	**Name**	**N**	**W**
1	El Tajo (Stream)	20° 40' 14.54585"	−103° 52' 23.13664"
2	Hacienda Labor de Rivera (Stream)	20° 40' 34.74973"	−103° 52' 43.36434"
3	Chapulimita (Stream)	20° 38' 29.49457"	−103° 52' 30.78224"
4	Cuisillos 1 (Stream)	20° 38' 21.74096"	−103° 47' 29.63054"
5	Cuisillos 2 (Stream)	20° 36' 31.04939"	−103° 48' 33.20593"
6	Acacias 1 (Stream)	20° 36' 21.19111"	−103° 49' 25.98805"
7	El Refugio (Stream)	20° 39' 49.59324"	−103° 44' 10.7637"
8	Teuchitlán River	20° 40' 50.02752"	−103° 50' 45.90186"
9	Teuchitlán (municipality discharge)	20° 40' 52.74163"	−103° 50' 40.197998"
10	Acacias 2 (Stream)	20° 36' 21.239557"	−103° 49' 26.06363"

Figure 1. Sampling site locations.

Agricultural, and Livestock Research INIFAP and Technological Institute José Mario Molina Pasquel y Henríquez, Academic Unit Arandas. They used sampling materials with the specific conditions and volume required for each evaluation parameter and the specifications for preservation and transfer to the laboratory. One-liter glass jars were adequately identified and labeled for handling with the site number and the name of the tributary. Sampling was conducted in August and December 2018. The data collected *in situ* were recorded on a format designed for this purpose.

The samples were stored in a cooler at a temperature of approximately 4 °C for their conservation and later transferred to the Microbiology Laboratory of the Technological Institute José Mario Molina Pasquel y Henríquez, Academic Unit Arandas, for analysis. It is essential to mention that, for the microbiological analysis, the samples were processed and analyzed within 24 hours of collection. For calcium, magnesium, sodium, carbonate, bicarbonate, and boron, the analysis was conducted at the Soil Fertility Laboratory in the Santiago Ixcuintla Experimental Field in Nayarit, which is part of the the National Institute of Forestry, Agricultural and Livestock Research (INIFAP). The analyzed parameters are shown in Table 2.

Sample analysis

The samples were analyzed according to the Mexican Official Norm, NOM-092-SSA1-1994, method for accountability of aerobic bacteria on plates; the Mexican Norm NMX-AA-42-1987, Water Quality-Determination of the Most Probable Number (MPN) of total coliforms, fecal coliforms (thermotolerant), *Escherichia coli* and Presumptive; and in the case of *Pseudomonas* spp. Calcium, sodium, lead, boron, and magnesium were analyzed using atomic absorption equipment.

Table 2. Analyzed parameters in the 10 main tributaries to La Vega Dam in Teuchitlán, Jalisco.

Parameters in water	Symbol	Units	Range(for irrigation) Ayers y Westcot (1994)
Salinity			
Electrical Conductivity	EC_w	$dS\ m^{-1}$	0-3
Total Dissolved Solids	TDS	$mg\ L^{-1}$	0-2000
Cations and anions			
Calcium	Ca^{++}	$Meq\ L^{-1}$	0-20
Magnesium	Mg^{++}	$Meq\ L^{-1}$	0-5
Sodium	Na^{+}	$Meq\ L^{-1}$	0-40
Carbonate	CO_3^{--}	$Meq\ L^{-1}$	0-.1
Bicarbonate	HCO_3^{-}	$Meq\ L^{-1}$	0-10
Boron	B	$Mg\ L^{-1}$	0-2
pH	pH	1-14	6.0-8.5
Microbiológical			
Total and fecal coliforms		100 mL	1000-2000
Escherichia coli		UFC	1000-2000
Pseudomonas spp.		UFC	0

Information Analysis

Descriptive statistics were performed on the results of the parameters evaluated *in situ*: pH, Temperature, Electrical Conductivity, Total Dissolved Solids, and Dissolved Oxygen. Once the calcium, magnesium, sodium, carbonate, and bicarbonate concentration were obtained, calculations were performed to determine the Sodium adsorption ratio (SAR) and residual sodium carbonate (RSC).

Sodium Adsorption Ratio (SAR) determination

For result analysis, the calculation of sodium absorption ratio (SAR) was used. The following equation calculates SAR:

$$SAR = \frac{\mathrm{Na}}{\sqrt{\frac{\mathrm{Ca} + \mathrm{Mg}}{2}}}$$

Where sodium, calcium, and magnesium are required in Meq L^{-1} from results in water analysis.

Residual Sodium Carbonate (RSC) determination

Residual Sodium Carbonate (RSC), to predict tendencies of calcium and magnesium soil precipitation and is calculated with the following equation:

$$RSC = \left(\mathrm{CO}_3^{2-} + \mathrm{HCO}_3^{1}\right) - \left(\mathrm{Ca}^{2+} + \mathrm{Mg}^{2+}\right)$$

Microbiological analysis

For the determination of Coliform bacteria (Most probable number technique), a) Presumptive test, b) Confirmatory test for Total Coliforms, and c) Confirmatory test for Fecal Coliforms *Pseudomonas* spp.: If there is microbial growth: Positive/g, if there is no microbial growth: Negative/g.

RESULTS AND DISCUSSION

For the parameters measured *in situ*, the following results were obtained: For pH, the values determined during the first sampling varied from 7.56 to 6.08, belonging to sampling sites number 1 "El Tajo" and number 8 "El Refugio," respectively, with an average of 6.8. Regarding the second sampling, the values were from 6.2 to 8.1, corresponding to sites number 4, "Cuisillos 1," and number 1, "El Tajo," with an average of 7.

Low pH can make toxic elements and compounds more mobile and available for uptake by aquatic organisms and plants. This can produce toxic conditions for aquatic life, particularly sensitive species (Yan *et al.*, 2007). According to CE-CCA-001/89, the pH values found in this research do not represent a risk for the protection of aquatic life, aquaculture, and irrigation water since they fall within the MPLs of 6-8.5 for irrigation water and 5-9 for drinking water.

The temperature recorded during the first sampling showed variability from 29.2 °C to 25.0 °C, values corresponding to sampling sites number 9, "Río Teuchitlán Descarga," and site number 3, "Arroyo Chapulimita," respectively, with an average of 26.3 °C. In the second sampling, the values obtained showed a variability ranging from 17.8 °C corresponding to sampling site number 1, "El Tajo," to 7.8 °C corresponding to site number 5, "Cuisillos 2", with an average of 12.9 °C. CE-CCA-001/89 does not mention LMP for temperature. Generally, the temperature obtained on the sampling dates does not represent a risk to aquatic life in La Vega Dam (Yan *et al.*, 2007; Custodio & Pantoja, 2012).

Electrical conductivity values ranged from 0.870 dS m^{-1} to 199 dS m^{-1}, results that were obtained at sampling sites number 7, "El Refugio," and site 9, "Río Teuchitlán (municipal discharge)," respectively, with an average of 0.410 dS m^{-1}. In the second sampling the values ranged from 0.900 dS m^{-1} to 0.200 dS m^{-1}, corresponding to sites number 4, "Cuisillos 1", and site number 8, "Río Teuchitlán," giving an average of 0.407 dS m^{-1}.

Electrical Conductivity (EC) is a key quality parameter that significantly influences crop productivity as the risk of salinity, measured as EC. The first effect that high electrical conductivity values have on crops is the inability of plants to compete for ions present in water and soil (physiological drought). EC CCA 001/89 mentions EC as Maximum Permissible Limit (MPL) of 0.1 dS m^{-1} for irrigation water; most of the sites where water samples were collected in this research presented values less than 0.1 dS m^{-1}.

Regarding Dissolved Oxygen, the range obtained in the first sampling was 5.02 mg L^{-1} to 1.48 mg L^{-1} values corresponding to sites number 3, "Arroyo Chapulimita," and site number 9, "Río Teuchitlán (municipal discharge)," with an average of 3.4 mg L^{-1}. During the second sampling, the observed range was 7.6 mg L^{-1} to 0.9 mg L^{-1}, corresponding to sites number 2, "Hacienda Labor de Rivera," and site number 4, "Cuisillos 1", respectively, with an average of 5.5 mg L^{-1}. EC-CCA-001/89 does not mention DO MPL for irrigation water. However, Gebremariam and Beutel (2008) say that low DO concentrations (less than 5 mg L^{-1}) can have critical consequences as they cause oxygen deficiency in plant roots, which can result in agronomic problems (Gebremariam and Beutel, 2008). According to the DO levels required by the crops mentioned in the literature, the DO determined in the various sampled sites, some of the sampled sites presented values that pose a risk to the crops (Gebremariam and Beutel, 2008).

Regarding Total Dissolved Solids, the range observed in the first sampling was from 480 mg L^{-1} belonging to site 10, "Acacias 2", to 105 mg L^{-1} corresponding to site 9, "Teuchitlán River (municipal discharge)" with an average of 251.4 mg L^{-1}. In the second sampling, a range was obtained from 450 mg L^{-1} corresponding to site 4, "Cuisillos 1", to 100 mg/L value obtained in sites 8 and 9 "Teuchitlán River" and "Teuchitlán River (municipal discharge),"respectively, with an average of 201 mg L^{-1}. CE-CCA--001/89 establish the MPLs for the concentration of TDS for the various water uses. For irrigation water, the MPLs mention 500 mg L^{-1}; therefore, most of the sampled sites are within these limits. Particles not ingested by aquatic organisms eventually settle, accumulating contaminants in the surface layers of the dam (Miller *et al.*, 2002; Bindler *et al.*, 2011).

Sodium Adsorption Ratio (SAR)

The average SAR for the two samplings is presented in Table 3. The sodicity risk in percentage was observed in that 50% of the sampled sites presented a low risk, 10% medium risk, 20% corresponded to high risk, and 20% presented a very high risk. The low sodicity risk corresponded to the 5 tributaries to La Vega Dam located in the north and west. Only one site presented medium risk (Cuisillos 2), while two sites presented high risk (Acacias 1 and 2), and two sites presented very high risk (Cuisillos 1 and El Refugio). It should be noted that the two sites that presented the highest SAR value correspond to the entrance of the El Salado River, which has been previously identified as having high salt content (De La Mora *et al.*, 2013). It is concluded that these salts have a geological origin since El Salado River originates in the Primavera forest, which has a volcanic origins.

The average SAR value was 12.43, with a standard deviation of 10.14. Sodium risk causes infiltration and permeability problems (Cuellar *et al.*, 2015; Can *et al.*, 2008). Although plant growth is initially limited by salinity levels (EC) present in irrigation water, the use of water with these characteristics (without sodium balance) under certain soil texture conditions can reduce production yield (Gasca *et al.*, 2011; Tartabull & Betancourt, 2016). The study area includes clay soils, which tend to salinize easily, according to GAT

Table 3. Results of sodicity risk in the tributaries to La Vega Dam.

Sodicity Risk (SAR) results		
SAR range	**Risk**	**Number of samples**
0-10	Low	5
11-17	Medium	1
18-26	High	2
Above 26	Very High	2

(2012). Also, a reduction in water infiltration can occur with large amounts of sodium with respect to calcium and magnesium (Dongli *et al.*, 2015).

Residual Sodium Carbonate (RSC)

RSC results showed that only 1 site (10%) presented a low risk, corresponding to a tributary in the northern part of the dam. The remaining 4 tributaries (40%) in this area offered a RSC value of less than 2, which indicates medium risk. However, the remaining 5 sites (50%) located in the eastern part of the dam presented a high risk (Table 4).

The RSC calculation results showed the highest value, 4.9, which corresponded to El Salado River. In contrast, the lowest value was 0.45, corresponding to a tributary in the northern part of the dam. The average RSC value found was 2.4, and the standard deviation was 1.33.

It is essential to mention that the sodium content in irrigation water negatively affects the soil's physical characteristics. When the sodium content is high compared to the content of other cations, there is a risk of soil deflocculation and loss of its physical characteristics such as granular structure, permeability, density, total porosity, consistency, water retention capacity (Zuñiga *et al.*, 2011. Therefore, determining SAR and RSC in irrigation water is crucial. The results indicated that 50% of the sampled sites showed a high risk of calcium and magnesium precipitation in soils irrigated with water from the tributaries to La Vega Dam.

Boron concentration obtained in both samplings

It was observed that the average in both samplings, in the 5 tributaries selected in the northern and western part of the dam, presented boron concentrations less than 1 mg L^{-1}. This level does not represent a risk for vegetation or aquatic life. However, the five sites presented concentrations between 4.5 and 11.2 mg L^{-1}. of boron, sites located east of the dam that correspond to El Salado River. These findings are consistent with those reported by Eisler (1990) in Eastern Europe, where high concentrations of boron found in surface waters are related to water with abundant minerals, indicating the natural origin of boron.

The maximum permissible limits (MPL) for boron (B) in irrigation water, as established by CE-CCA-001/89, specify that for sensitive crops such as peas, onions and avocados (Ayers and Wescot, 1994), among others, the water must contain a maximum of 0.75 mg L^{-1}, except for other crops such as corn, squash and oats (Ayers and Wescot, 1994) among others where concentrations of up to 3 mg L^{-1} can be accepted. The average B value found in this investigation was 4.02 mg L^{-1}. It was observed that this value exceeds 5 times the MPL. The average of the highest values determined in 5 sampling sites exceeds 2.5

Table 4. Results of the Residual Sodium Carbonate (RSC) risk in the tributaries to La Vega Dam.

RSC (Me L^{-1})	**Risk**	**Number of samples**
<1.25	Low	1
1.25-2.5	Medium	4
Above 2.5	High	5

times the concentration applicable to tolerant crops. The standard deviation of the data corresponded to a value of 4.18.

Microbiological analysis showed contamination at all sampling sites. In this regard, it is essential to mention the activities carried out in the area influencing the analyzed tributaries. For example, in the northern part, where the Teuchitlán River is one of the main tributaries to the dam, two samples were collected in this river, one before receiving the domestic discharges from the city of Teuchitlán and the other after receiving the discharges. Fecal contamination was expected at the site after the discharge but not at the sampling site before the discharge, where contamination was likely to be present but not at the level found.

Also, in the northern part of the dam, the site that corresponds to el Tajo, this river is formed from springs in the area and surface runoff from agricultural lands during the rainy season. Given the origin of the river, minimal microbiological contamination was expected to be found; however, the results showed countless fecal contaminations and positive for *Pseudomonas* spp. It is important to mention that the population closest to these springs, where the river originates, is approximately five kilometers from it, and physically, no contamination from discharges or characteristic odor of wastewater discharges were detected. Therefore, the origin of the fecal contamination is unknown.

Another tributary in the eastern part of the dam is the Chapulimita Stream, where a characteristic odor and color of wastewater were detected. This stream originates in the upper part of the basin, where some communities are located, such as Ahualulco del Mercado. It is unknown whether domestic wastewater is discharged directly. However, according to the results obtained, it is evident that there are discharges before entering La Vega Dam.

Another runoff originates in the Hacienda Rivera northeast of the dam (positive in the two analyses carried out in this work) and presents color and odor. There is no information on domestic discharges; however, the runoff is located a few meters from a hotel, which may be a fixed source of fecal contamination.

In the southeastern part, samples were collected from sites identified as Acacias 1 and Acacias 2. The selected sites correspond to a sample before a residential center identified with the same name (Acacias) and another after. The results were positive for both total and fecal coliforms and *Pseudomonas* spp. cases.

Also, in the southeast, the sites identified as Cuisillos 1 and Cuisillos 2 tested positive for the studies. In this area, there is a community with the same name, Cuisillos, so it is believed that sewage is also discharged into this river. Finally, the sampling site identified as El Refugio in the eastern part of La Vega Dam also tested positive for fecal and total coliforms and for *Pseudomonas* spp. It is important to mention that the results were expected since a few kilometers from this site is the city of Tala, Jalisco, and it is known that domestic discharges are released into the river. It is also known that the discharges from the sugar mill in Tala, Jalisco, also pour their waste into this river, in addition to the runoff typical of the rainy season, making this river (El Salado) one of the primary sources of pollution for La Vega Dam, not only from fecal contamination but from other types of contaminants reported in the literature.

CONCLUSIONS

According to the MPL mentioned in the Ecological Criteria for Water Quality CE-CCA-001/89, parameters such as pH, EC, and TDS are within the MPL for use in aquaculture, irrigation, domestic and recreational use. In the case of DO, some values fall below the MPL, which is 5 mg L^{-1}. The most significant contamination contribution to La Vega Dam was detected in the eastern part of the dam, with the entrance of El Salado River and other minor tributaries. Among the parameters analyzed, sodium presented high concentrations, contributing to a high value of both the SAR and the RSC. High concentrations of boron were also observed, which can negatively affect crops in the area according to their sensitivity. In addition to *Pseudomonas*, a high concentration of total and fecal coliforms was also found, which indicates fecal contamination. On the other hand, the northern and western parts of the dam presented a low risk in the SAR and RSC values. However, the total and fecal coliforms are outside the maximum permissible limits of the Mexican Official Norm cited, and the presence of *Pseudomonas* spp. Limiting its use for irrigation water. Based on the above, the water quality of the main tributaries to La Vega Dam on the east side is unsuitable for irrigation use. Constant monitoring of the tributaries to La Vega dam and within the reservoir is recommended. Additionally, wastewater treatment plants in various municipalities around La Vega Dam, which can reduce pollution, are recommended.

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Colegio de Postgraduados

Forest Management and Tree Species Diversity in a Temperate Forest of the Meseta Purhépecha, Michoacán

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ABSTRACT

Objective: To evaluate how traditional forest management practices impact the diversity and structure of temperate forests in the Nuevo San Juan Parangaricutiro Indigenous Community (ICNSJP), with the goal of providing information on the sustainable management of these forests.

Design/methodology/approach: Forest inventory data were collected and analyzed in stands treated with four thinning (A4) through the Silvicultural Development Method (SDM) and contrasted with stands that have not been intervened for more than 30 years (A0). Diversity indices and stand variables were used to statistically compare the effects of the treatments.

Results: In total 20 species were recorded, 13 in A0 and 18 in A4. The true diversity (q1) recorded a value of 3.3±1.6 in A4 and 1.9±0.8 in A0, although this difference was not statistically significant. The species with highest importance value were *Pinus douglasiana* Martinez (30%) for A0 and *P. pseudostrobus* Lindl (27%) for A4.

Limitations on study/implications: According to the SDM, the cutting cycle in A4 has not yet ended since the release and regeneration treatments are missing, therefore, monitoring is recommended to evaluate the effect of the SDM more comprehensively on diversity and structure in the forests of the ICNSJP.

Findings/conclusions: Forest management in the ICNSJP has shown a positive effect in terms of species diversity and due to its commercial value, the development of *P. pseudostrobus* has been favored. In general, forest management has contributed to socioeconomic development by promoting species diversity, a key element for sustainable development.

Keywords: SDM; *Pinus pseudostrobus*; *Pinus douglasiana*; Forest thinning; Pretzsch A index.

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INTRODUCTION

Forest management practices impact the diversity and composition of tree species. The degree of impact depends on the intensity of the treatment and the successional stage of the vegetation. Clear-cutting, which involves the removal of all trees, tends to reduce species diversity (Guevara-Fisher *et al.*, 2021), whereas selection treatments or low-intensity

interventions have shown a positive effect on species diversity and composition (Monarrez-Gonzalez *et al.*, 2020). Understanding the impact of these treatments on forest diversity and structure is essential for developing management proposals that ensure conservation, restoration, and appropriate utilization actions in managed forests.

Forest management aims to optimize the utilization of forest resources to meet current needs without compromising the provision of goods and services for future generations (Aguirre-Calderón, 2015). In this context, sustainable forest management is essential for conserving biodiversity, ecosystem values, and landscapes (Hernández-Salas *et al.*, 2013). Consequently, the structure and diversity within an ecosystem serve as indicators of the effects of silvicultural practices and sustainable forest regimes (Han *et al.*, 2022; Xi *et al.*, 2021). Forest management impacts the structure and diversity of natural forests (Corral-Rivas *et al.*, 2005), generally reducing species diversity and abundance by favoring only some (Silva-García *et al.*, 2021).

One way to evaluate the effect of silvicultural treatments is using diversity and structure indices in areas that have been managed and comparing them with stands with little or no intervention (González-Fernández *et al.*, 2022; Hernández-Salas *et al.*, 2013; Jiménez-Pérez & Aguirre-Calderón, 2001; Xi *et al.*, 2021). These indices have been used in various studies to evaluate the impact of forest harvesting on tree species in a forest (Corral-Rivas *et al.*, 2005; Hernández-Salas *et al.*, 2013; Jiménez-Pérez & Aguirre-Calderón, 2001; Návar-Cháidez & González-Elizondo, 2009; Silva-García *et al.*, 2021), to measure differences over time and space (Ramírez-Guaman & Lozano, 2024), as well as to monitor changes caused by different silvicultural treatments or to define appropriate practices that lead to good forest management (Vásquez-Cortez *et al.*, 2018).

Although biodiversity has been extensively researched in other regions, there is a knowledge gap regarding tree diversity and composition in the temperate ecosystems of the Purhépecha Plateau in Michoacán. In this area, timber harvesting has been carried out using the Silvicultural Development Method (SDM) for more than 35 years. The Indigenous Community of Nuevo San Juan Parangaricutiro (ICNSJP) holds Forest Stewardship Council (FSC) certification due to its forest management practices that adhere to official regulations and international standards, enabling them to access the international market for their products (CONAFOR, 2022; Velázquez *et al.*, 2001).

The objective of this study was to evaluate the effect of traditional forest management on the diversity and structure of temperate forests within the ICNSJP. This aims to provide scientific support for decision-making directed towards sustainable forest management in forest communities.

MATERIALS AND METHODS

Study Area

The study was conducted on the Purhépecha Plateau in the state of Michoacán, specifically in the Indigenous Community of Nuevo San Juan Parangaricutiro (ICNSJP) (Figure 1). The forests in this area have been historically managed for the utilization of both timber and non-timber forest products. The ICNSJP is located in the physiographic region of the Trans-Mexican Volcanic Belt and the southwestern end of the Purhépecha

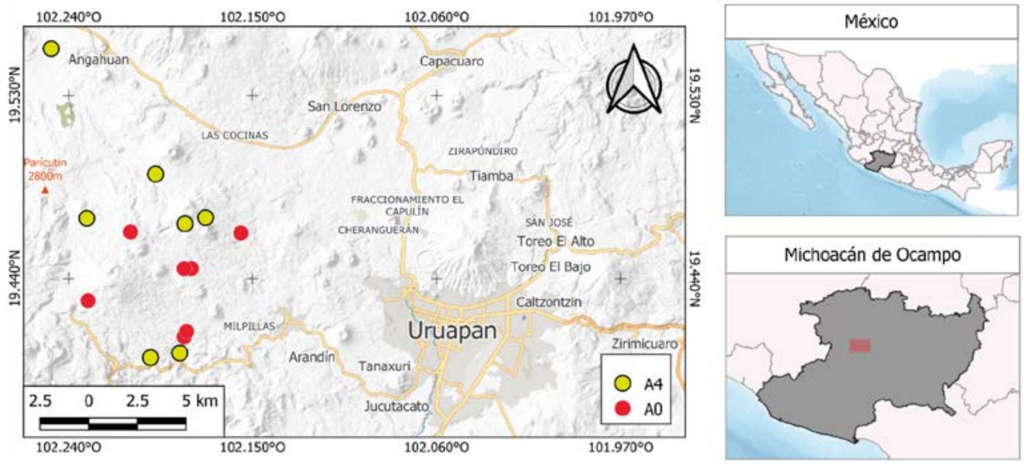

Figure 1. Sampling sites in the Indigenous Community of Nuevo San Juan Parangaricutiro (ICNSJP) in the state of Michoacán.

Sierra. The climate is temperate humid $C(w_2)(b)$ with summer rainfall $C(w_2)(w)$, and the area features rugged topography with slopes ranging from 5% to 80%, at elevations from 1,900 meters above sea level (forestry technical principal building of ICNSJP) to 2,920 meters above sea level (fire tower a Cerro of Pario). Annual precipitation ranges between 1,500 and 2,000 mm, with an average annual temperature of 12 to 14 °C. The soils are of volcanic origin, including medium-textured humic andosols, coarse-textured ochric andosols, coarse-textured dystrophic regosols, and medium-textured haplic faeozems (Bello-González *et al.*, 2015).

The area under forest management is 10,653 ha^{-1}, of which 59% of the total area is dedicated to timber production through the SDM. The vegetation is primarily represented by pine forest, pine-oak forest, pine-fir forest, and montane mesophyll forest, with notable species including *Pinus pseudostrobus* Lindl., *P. montezumae* Lamb., *P. leiophylla* Schl. & Cham., *P. devoniana* Martínez., *P. douglasiana* Martínez, *Abies religiosa* (HBK) Schltdl. & Cham., *Quercus rugosa* Née., *Q. obtusata* Humb. & Bonpl., *Q. laurina* Humb. & Bonpl., *Q. castanea* Née., *Q. candicans* Née., *Q. dysophylla* Benth., *Alnus jorullensis* subsp. lutea Furlow., *Carpinus caroliniana* Walter., *Tilia mexicana* Schltdl., and *Ternstroemia pringlei* (Rose) Standl (Bello-González *et al.*, 2015).

Data Collection

Two forest strata with stands under management in areas belonging to the ICNSJP were selected. One stratum, which has not received any silvicultural treatment for more than 30 years, was designated as A0, and the other, where four thinning operations (based on the SDM) have been applied, was designated as A4. In each of these areas, seven random sampling sites of 1000 m^2 were established, where data for forest inventories were collected, including diameter at 1.30 meters measured with a diameter tape ($d_{1.3}$, cm), total height obtained with a Forestry PRO II Nikon® laser hypsometer (*h*, *m*), and canopy diameter measured in two projections (N-S and E-W) with a diameter tape (CD, m). Each tree species name was also recorded.

Information Analysis

Species diversity was characterized using the Importance Value Index (*IVI*) and the estimation of diversity indices, such as Shannon's Index (*H*'), its transformed version commonly known as Hill numbers (q1) or true diversity, and the vertical distribution index of species (A) (Table 1). To determine the *IVI* of the species, parameters such as relative abundance (*Ra*), estimated based on the number of trees per hectare (N ha^{-1}), relative dominance (*Rd*), which is obtained as the proportion of basal area (G ha^{-1}), and relative frequency (*Rf*), which is the number of sites where the species occurs, were used (Mostacedo & Fredericksen, 2000). Based on these three (*Ra*, *Rd*, and *Rf*), the Importance Value Index (*IVI*) was calculated to hierarchically rank the dominance of each taxon, in relative values from 0 to 100.

Vertical composition was analyzed using the vertical species distribution index (A) or Pretzsch Index (Pretzsch, 2009; Rubio-Camacho *et al.*, 2014) (Table 1). To determine this index, three height zones were defined: Zone I (80 to 100% of maximum height); Zone II (50 to 80% of maximum height); and Zone III (0 to 50% of maximum height) (Pretzsch, 2009).

In addition to the diversity indices, stand variables were also estimated per site to enable statistical comparison between areas. Among the most important variables are average height (*h*) and basal area per hectare (G ha^{-1}). Their importance lies in their role as indicators of the distribution of dimensions and the density of the stands.

To compare the results between the evaluated areas, the Student's T-test was used, with Welch's modification to correct for heteroscedasticity. The assumption of normality was verified using the Shapiro-Wilk test, and when this assumption was not met, the Wilcoxon test was used for contrasts. All statistical analyses and normality tests were performed using R software version 4.1.2 (R Core Team, 2021).

RESULTS AND DISCUSSION

In the 14 sampling sites, a total of 20 species were recorded, distributed across 8 families. Pinaceae and Fagaceae represented the highest percentage of species (30% each), followed by Betulaceae and Pentaphylacaceae with 10% each. Overall, A4 had a higher number of

Table 1. Equations for Analyzing the Structure and Diversity of the Temperate Forest of the ICNSJP, Michoacán.

Index	Equation	Definitions
Shannon's entropy index	$H' = -\sum_{i=1}^{S} p_i * \ln(p_i)$	S=Number of species P_i=Abundance proportion of the *i*-th species
True diversity index, based on Shannon's entropy	$q1 = \exp(H')$	
Species vertical distribution index (A)	$A = -\sum_{i=1}^{S}\sum_{j=1}^{Z} p_{ij} * \ln p_{ij}$	Z=number of vertical zone; p_{ij}=proportion of the *i*-th species in the *j*-th zone, $p_{ij}=n_{ij}/N$; where n_{ij} is the number of records of the *i*-th species in the *j*-th zone and N is the total number of records.

species with 18, while A0 recorded 13. Finally, of the 20 species recorded, 11 were found in both areas, 7 were present only in A4, and 2 were found only in A0 (Table 2).

Stand Diversity and Structure

Regarding the statistical contrasts of diversity and structure, it was found that the index A did not meet the normality assumption ($p<0.05$), while the others did (the distribution does not differ from a normal distribution). On average, considering the seven sites per area, species richness was recorded as 3 ± 1.2 and 5.7 ± 3.3 for A0 and A4 respectively, although this difference was not statistically significant ($P>0.05$). A similar pattern was observed with true diversity, as A4 had higher values (3.3 ± 1.6), but the difference with A0 (1.9 ± 0.8) was not significant ($p>0.05$). The same occurred when comparing the vertical distribution of species, as index A had an average of 1.2 ± 0.5 and 1.7 ± 0.5 for A0 and A4, without significant differences ($p>0.05$). These results are relevant because, in general terms, sites with repeated thinning showed a positive effect on species diversity. On one hand, diversity did not decrease, and on the other hand, more species were found in A4 even though the average per site was not significant (Table 2).

The obtained results are similar to others in which species composition in pine-oak forests was studied (Ávila-Sánchez *et al.*, 2018). It has been documented that species richness

Table 2. Families and Species Present in the Temperate Forest of the Indigenous Community of Nuevo San Juan Parangaricutiro, Michoacán.

Family	Species	Common name	Records	
			A0	A4
Betulaceae	*Alnus acuminata* Kunth	Tepamo	0	1
	Alnus jorullensis Humboldt, Bonpland & Kunth	Aile	5	1
Clethraceae	*Clethra mexicana* DC.	Cletra	0	5
Ericaceae	*Arbutus xalapensis* Kunth	Madroño	1	2
Fagaceae	*Quercus candicans* Schltdl. & Cham	Encino ancho	1	2
	Quercus castanea Née.	Encino capulincillo	1	12
	Quercus crassipes Bonpl.	Encino colorado	0	1
	Quercus laurina Bonpl.	Encino laurelillo	13	30
	Quercus obtusata Bonpl.	Encino blanco	2	23
	Quercus rugosa Née.	Tocuz	2	39
Pentaphylacaceae	*Cleyera integrifolia* (Benth.) Choisy	Tchcari-charapiti (lengua purépecha)	0	1
	Ternstroemia lineata DC.	Tila	0	43
Pinaceae	*Pinus devoniana* Lindl.	Pino escobeton	20	0
	Pinus douglasiana Mtz.	Pino chino	77	1
	Pinus leiophylla Schiede ex Schltdl. & Cham.	Ocote chino	13	19
	Pinus montezumae Lamb.	Pino lasio	1	36
	Pinus pseudostrobus Lindl.	Canis-pino blanco	37	73
	Pinus teocote Schiede ex Schltdl	Tso-arza (lengua purépecha)	16	0
Salicaceae	*Salix paradoxa* Kunth	Cucharillo	0	1
Symplocaceae	*Symplocos citrea* Lex. ex La Llave & Lex.	Limoncillo	0	6
Total			189	296

and diversity values are related to the type of vegetation, especially in temperate forests of Mexico, which are associated with the altitudinal range where the genera *Pinus* and *Quercus* generally dominate (Ávila-Sánchez *et al.*, 2018; Ubaldo-Velasco *et al.*, 2023). Additionally, various anthropogenic factors have shaped their structure and species composition over time, particularly in temperate forests with forest management.

In the study area, the tree communities are generally dominated by the Pinaceae family in the tree stratum. However, in the lower parts of the forests of the ICNSJP, the *Pinus* forests mix with species of *Quercus*, *Alnus*, and *Arbutus*. These results are similar to those reported for unmanaged temperate forests in other states such as Durango, Chihuahua, Puebla (Caballero-Cruz *et al.*, 2022), and Nuevo León (Manzanilla-Quijada *et al.*, 2020).

The structure, analyzed through stand attributes, showed significant differences. The basal area per hectare had a higher average in A0 (24.6 ± 3.5 m^2) compared to A4 (19.7 ± 3.4 m^2) ($t=2.63$, $df=11.99$, $p=0.021$). A similar pattern was observed with average height, where A0 recorded 24.3 ± 7.99 m and A4 15.4 ± 4.04 m ($t=2.65$, $df=8.87$, $p=0.027$). Additionally, the mean quadratic diameter also shows these differences, with A0 at 37.7 ± 11.6 cm and A4 at 26.1 ± 7.01 cm ($t=2.27$, $df=8.89$, $p=0.046$). This indicates that, on average, trees are larger in A0 and that, in general, this area has a greater basal area, which is related to the conditions of forest mass density.

Importance Value Index by Species

Generally, for A0, the species with the highest importance values was *P. douglasiana*, as it recorded 110 N ha^{-1}, 8.4 G ha^{-1}, and was present in three of the seven sampling sites. The second most important species was *P. pseudostrobus*, while the third was *P. devoniana*. On the other hand, the species with the lowest importance values were *Q. castanea*, *Q. candicans*, and *A. xalapensis* (Table 3). On the other hand, A4 *P. pseudostrobus* was the most important, recording 104 N ha^{-1}, 8 G ha^{-1}, and was present in six of the seven sites. The second most important species was *P. montezumae*, while the third was *Q. rugosa*. The species with the lowest importance values were *A. acuminata*, *S. paradoxa*, and *Q. crassipes* (Table 3). This analysis revealed a difference in the composition of the most important species, highlighting oaks, which are becoming more relevant, such as the species *Q. rugosa*.

Regarding vertical distribution, the maximum height recorded for A0 was 43.9 m and for A4 was 39.9 m, which corresponds to 100% according to Pretzsch's (2009) methodology. In general, it was observed that both in A0 and A4, the species with the greatest height were *P. montezumae* and *P. pseudostrobus*, being the only species found in zone I. However, in both areas (A0 and A4), *P. pseudostrobus* dominates in basal area and number of recorded individuals (Table 4). These results are consistent with the heights recorded in temperate forests in northern Mexico (Rubio-Camacho *et al.*, 2014; Silva-García *et al.*, 2021) and with the work of Ubaldo-Velasco *et al.* (2023), which reports species such as *P. pseudostrobus* and *Q. rugosa* with the highest importance value index (23.3% and 14.3%, respectively) in the upper stratum of a temperate forest in Oaxaca.

For zone II of A0, four species of *Pinus* were found, whereas in A4, four species of *Pinus* and three species of *Quercus* were found, indicating greater competition from tolerant species. Another notable difference is that in A0, the species with the largest basal area and

Table 3. Importance Value Index (*IVI*) by Species in 14 Sampling Sites in Natural Forests of the Indigenous Community of Nuevo San Juan Parangaricutiro, Michoacán.

A0					A4				
Species	**Ra**	**Rd**	**Rf**	**IVI**	**Species**	**Ra**	**Rd**	**Rf**	**IVI**
P. douglasiana	40.7	34.2	14.3	29.7	*P. pseudostrobus*	24.7	40.6	15.0	26.8
P. pseudostrobus	19.6	41.4	19.0	26.7	*P. montezumae*	12.2	22.5	5.0	13.2
P. devoniana	10.6	6.9	4.8	7.4	*Q. rugosa*	13.2	7.0	12.5	10.9
Q. laurina	6.9	5.4	9.5	7.3	*P. leiophylla*	6.4	12.6	7.5	8.8
P. leiophylla	6.9	4.7	9.5	7.0	*T. lineata*	14.5	3.5	7.5	8.5
P. teocote	8.5	4.5	4.8	5.9	*Q. laurina*	10.1	5.8	7.5	7.8
Q. rugosa	1.1	0.1	9.5	3.6	*Q. obtusata*	7.8	2.4	5.0	5.1
A. jorullensis	2.6	0.9	4.8	2.8	*Q. castanea*	4.1	1.3	5.0	3.5
Q. obtusata	1.1	0.5	4.8	2.1	*C. mexicana*	1.7	0.4	7.5	3.2
P. montezumae	0.5	0.6	4.8	2.0	*S. citrea*	2.0	1.3	5.0	2.8
Q. castanea	0.5	0.5	4.8	1.9	*Q. candicans*	0.7	0.9	5.0	2.2
Q. candicans	0.5	0.2	4.8	1.8	*A. xalapensis*	0.7	0.3	2.5	1.2
A. xalapensis	0.5	0.1	4.8	1.8	*P. douglasiana*	0.3	0.6	2.5	1.2
					C. integrifolia	0.3	0.3	2.5	1.0
					A. jorullensis	0.3	0.2	2.5	1.0
					A. acuminata	0.3	0.1	2.5	1.0
					S. paradoxa	0.3	0.1	2.5	1.0
					Q. crassipes	0.3	0.0	2.5	1.0

Where: Ra=Relative Abundance, Rd=Relative Dominance, and Rf=Relative Frequency.

greatest number of recorded individuals was *P. douglasiana*, whereas in A4, *P. pseudostrobus* had the highest values (Table 4). This could be related to the results of the silvicultural actions applied in A4, which are assumed to have favored the development of *P. pseudostrobus*, while in A0, *P. douglasiana* is gaining prominence in stand development. This is explained by the commercial importance of the species in the region, as the wood of *P. pseudostrobus* is marketed on a larger scale than that of *P. douglasiana* (CONAFOR, 2022).

Zone III exhibited the greatest species richness, with 12 species recorded in A0 and 17 in A4. Once again, a dominance of *P. douglasiana* in A0 and *P. pseudostrobus* in A4 is observed (Table 4). This is consistent with findings by García-García *et al.* (2019) for a Pine-Oak forest in Chihuahua, where they report that in the forest management area, the taxa *Pinus* and *Quercus* were dominant, and similarly, most species were found in zone III. These observations align with other studies in temperate forests (Rubio-Camacho *et al.*, 2014).

According to Lamprecht (1990), some species exhibit a continuous vertical distribution, as seen with *P. pseudostrobus* in this study. In this sense, the *Pinus* taxon might ensure its persistence in the composition and structure of the forest since it is present in all altitude zones (García-García *et al.*, 2019). On the other hand, this genus also dominates in terms of basal area (G ha^{-1}), which is considered an indicator of a forest's capacity to produce biomass (Mora, 2022).

Table 4. Vertical Distribution of Three Zones in the Natural Forest of the Indigenous Community of Nuevo San Juan Parangaricutiro, Michoacán.

A0					**A4**				
Zone	**Species**	**N**	**h (sd)**	**G ha^{-1}**	**Zone**	**Species**	**N**	**h (sd)**	**G ha^{-1}**
I	*P. montezumae*	1	35.7 (-)	0.2	I	*P. montezumae*	6	33.5 (0.7)	1.8
	P. pseudostrobus	**22**	**37.6 (2.1)**	**7.5**		***P. pseudostrobus***	**7**	**36.1 (2.3)**	**2.9**
II	*P. devoniana*	7	24.3 (3.4)	1.0	II	*P. douglasiana*	1	24.3 (-)	0.1
	P. douglasiana	**33**	**23.9 (1.8)**	**4.6**		*P. leiophylla*	12	25.4 (3)	2.2
	P. leiophylla	4	28.6 (4.3)	0.7		*P. montezumae*	16	25.1 (2.6)	2.3
	P. pseudostrobus	12	32.6 (3.4)	2.6		***P. pseudostrobus***	**19**	**24.8 (2.6)**	**3.7**
III	*A. jorullensis*	5	9.5 (2.6)	0.2		*Q. candicans*	1	26.9 (-)	0.1
	Arbutus xalapensis	1	12.1 (-)	0.0		*Q. laurina*	1	22.6 (-)	0.1
	P. devoniana	13	15.3 (4.2)	0.7		*Q. rugosa*	2	24.3 (0.3)	0.3
	P. douglasiana	**44**	**20.2 (2.1)**	**3.8**	III	*A. acuminata*	1	9.5 (-)	0
	P. leiophylla	9	15.9 (5.2)	0.4		*A. jorullensis*	1	15.2 (-)	0
	P. pseudostrobus	3	13.9 (1.7)	0.1		*A. xalapensis*	2	13 (2.3)	0.1
	P. teocote	16	11.1 (4.4)	1.1		*C. mexicana*	5	9.5 (1.6)	0.1
	Q. candicans	1	16.8 (-)	0.0		*C. integrifolia*	1	16.9 (-)	0.1
	Q. castanea	1	18.2 (-)	0.1		*P. leiophylla*	7	11.4 (5.5)	0.3
	Q. laurina	13	14.4 (4.4)	1.3		*P. montezumae*	14	11.9 (3.7)	0.3
	Q. obtusata	2	14.2 (0.1)	0.1		***P. pseudostrobus***	**47**	**11.9 (3.8)**	**1.4**
	Q. rugosa	2	7 (1)	0.0		*Q. candicans*	1	10.8 (-)	0
						Q. castanea	12	8.4 (3.8)	0.3
						Q. crassipes	1	6.6 (-)	0
						Q. laurina	29	12.2 (3.9)	1.1
						Q. obtusata	23	8.2 (2.9)	0.5
						Q. rugosa	37	10.1 (4.2)	1.1
						S. paradoxa	1	7.2 (-)	0
						S. citrea	6	11.9 (2.6)	0.3
						T. lineata	43	8.9 (2.3)	0.7

Where: N=number of individuals, h=total height (m), G ha^{-1}=basal area.

The results obtained align with findings from other studies, indicating that the decrease in tree diversity and the lower number of individuals in vertical zones I and II in temperate forests in Mexico are associated with silvicultural interventions, as well as natural growth and competition among species (García-García *et al.*, 2019; Ramírez-Guaman & Lozano, 2024; Ramírez-Santiago *et al.*, 2019; Rendón-Pérez *et al.*, 2024; Silva-García *et al.*, 2021). These results also support the hypothesis that managed areas differ from each other, even under similar edaphoclimatic conditions, due to the various silvicultural treatments applied during the forest rotation period (García-García *et al.*, 2019; Han *et al.*, 2022; Hernández-Salas *et al.*, 2013; Ramírez-Santiago *et al.*, 2019; Xi *et al.*, 2021). Therefore, both natural and secondary managed forests undergo changes in their composition, structure, and functions due to forest management (Ramírez-Guaman & Lozano, 2024).

In general, forest management in temperate forests is complex with regard to the conservation of tree species diversity and composition (Rendón-Pérez *et al.*, 2024; Rubio-Camacho *et al.*, 2014). This is partly because one of the objectives of the Silvicultural Development Method (SDM) is the regeneration of even-aged and monospecific stands, primarily favoring pine species due to their commercial value in the market. Therefore, forest management strategies need to be objective (Aguirre-Calderón, 2015) and take a holistic approach to ensure the sustainable management of a temperate forest, such as that of the Indigenous Community of Nuevo San Juan Parangaricutiro (ICNSJP). Finally, this study demonstrates that species diversity has actually been improved by the silvicultural treatments applied in A4, as no differences were found compared to the unmanaged stands. However, the dominance of certain species, such as *P. pseudostrobus*, is attributed to the objectives set during the application of the Silvicultural Development Method (SDM) and its economic importance.

CONCLUSIONS

The natural forests of ICNSJP are dominated by the genera *Pinus* and *Quercus*, with specific variations in species predominance according to the treatment. The results indicate that the applied treatments have not negatively affected species diversity, which is considered a positive outcome for ecosystem conservation. However, the greater dominance of *P. pseudostrobus* in treatment A4 could have important commercial implications, given the economic value of this species. This result suggests that certain treatments may be used to favor commercially important species without compromising the overall diversity of the forest. According to the SDM, the cutting cycle in A4 is not yet complete as the release and regeneration treatments; therefore, it is recommended to continue monitoring to more comprehensively evaluate the effect of SDM on diversity and structure in ICNSJP forests.

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Colegio de Postgraduados

Physical-chemical and nutritional parameters of liquid porcine effluent from a biodigester supplemented with a lagoon system

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ABSTRACT

Objetive: Determining the concentration of physical-chemical and nutritional parameters of the wastewater derived from a biodigester complemented with lagoon train.

Metodology: A study was carried out in the CEAJAL livestock production module, 12 gestating sows, fresh solid excreta was collected manually (shovel and wheelbarrow), the lagoon-type biodigester was fed daily with two levels of organic load (CO), CO5% and CO15%. The biogas and wastewater were evaluated in four periods of 40 days each. The Influent washing water (INF) entered the biodigester, then the liquid effluent (EFL) was subjected to complementary treatment of EFL stabilization pits, Pit 2, Pit 3 and Pit 4, determining physical-chemical parameters such as TSS, pH, CTE and COD, and nutritional parameters such as NT and FT. The data were analyzed using descriptive and differential statistics.

Results: The methane content in the biogas was 59.8%; CO5% and 60.2%; CO15% ($p>0.05$). The physical-chemical parameters of INF such as SST ml/L was 67.4; CO5% and 81.3; CO15%. EFL was 23.2 and 48.0, respectively, in COD ml/L of INF was 738.7; CO5% and 1807.7; CO15%. EFL was 1444.2 and 2522.5, respectively, in NT ml/L of INF was 128.3; CO5% and 111.9; CO15%. EFL was 436.9 and 554.6, respectively.

Conclusions: Despite a lower CO, methane production is in the normal range and the physical-chemical and nutritional parameters of the wastewater as it passes into stabilization lagoons can be taken as a reference to determine the CO that should enter to the biodigester with the purpose of providing complementary treatment of the wastewater generated in pig farms.

Keywords: wastewater, pig farm, technological adoption.

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INTRODUCTION

Biodigesters have been used on pig farms for biogas production and, in the last decade, have served as a wastewater treatment method (Cubillos-Sierra *et al.*, 2018). The efficiency of a biodigester used for wastewater treatment can be determined by the concentration of physical-chemical and nutritional parameters. In recent years, microbiological aspects have also been considered (Penafiel *et al.*, 2021). However, in many cases, the levels of Organic Load (OL), Hydraulic Retention Times (HRT), and the complementary treatments of each of the byproducts derived from a biodigester are omitted (Galindo-Barboza *et al.*, 2020).

Pigs in confinement farms generate different types of organic waste. Regardless of the level of farm technology, these wastes represent a contamination risk due to the lack of utilization of all the nutrients consumed in their feed ration. The quantity and quality of excreta depend on the diet, animal age, and facility design (Kebreab *et al.*, 2016). Therefore, the accumulated excreta used in crop field fertilization without prior treatment has led to increased levels of nitrogen and phosphorus in the soil. Additionally, since most of the piles or pits containing manure on farms are exposed to the open air, rain and wind dissolve the soluble nutrients. At the same time, ammonia emissions into the environment increase, resulting in a stronger odor, as well as the infiltration of nitrites and nitrates into nearby water bodies, a process known as eutrophication (Pinos-Rodríguez *et al.*, 2012; Domínguez-Araujo *et al.*, 2023).

The establishment of a biodigester should be considered within an integrated organic waste management program, which helps mitigate environmental damage and benefits large, medium, and small farms in environmental, technological (Magnusson *et al.*, 2022), and economic (Durante-Mühl & De Oliveira, 2022) aspects.

An integrated waste management program, as a technological adoption, must consider the separation and classification of the material to be treated (solid and liquid waste) to obtain the maximum benefit from the processes (Barrera-Cardoso *et al.*, 2020). Additionally, this facilitates the selection of the process and the implementation of practical methodology (Somagond *et al.*, 2020).

The management and treatment of liquid excreta through biodigesters should be combined with other strategies such as physical, chemical, and even biological processes that are easy to adopt, compatible with biodigesters, and reasonably priced. Working synchronously, these strategies generate byproducts (organic fertilizers, biogas, and treated wastewater) for use both within and outside the farms (Domínguez-Araujo *et al.*, 2023).

Therefore, the objective of this study was to determine the physical-chemical and nutritional parameters of the liquid effluent from a biodigester fed with different organic loads, using stabilization ponds as a complementary treatment.

MATERIALS AND METHODS

Location

This study was conducted in the pig production and waste utilization module at the Central Highlands of Jalisco Experimental Field of INIFAP, in Tepatitlán de Morelos, Jalisco.

Biodigester

An anaerobic lagoon-type biodigester with a capacity of 6 m^3 was used, operating at a mesophilic temperature (32 °C) outside and maintaining an average temperature (24 °C) inside. It has a continuous flow feeding regime that aids agitation inside by gravity and pressure, with a Hydraulic Retention Time (HRT) of 30 days. Adjacent to the biodigester is the discharge tank (effluent), followed by the lagoon system, consisting of three adjacent pits for the post-treatment of the liquid effluent. This liquid undergoes decantation through the pits, with an HRT of 7 days in each pit (Figure 1).

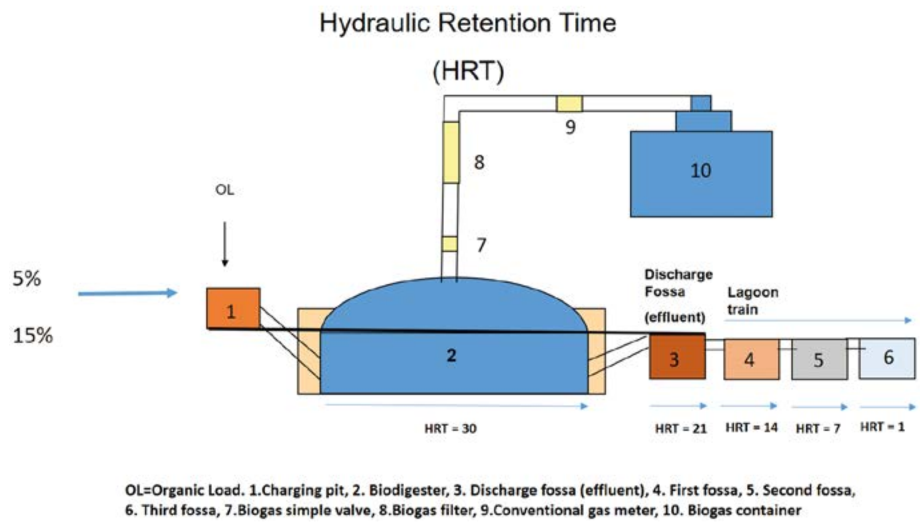

Figure 1. Schematic of the biodigester with three adjacent stabilization ponds (Image modified from Domínguez-Araujo *et al.*, 2023).

Organic Loads

From the gestation area, a total of 12 sows in production with an average weight of 220 kg, 1.8 kg of solid organic waste was excreted per day on average. These were manually collected (using a shovel and wheelbarrow) to obtain fresh excreta each day.

Once the solid excreta were removed from the pens, they were weighed to determine the organic load (OL) percentages. The excreta were placed in a 100-liter cylinder (the maximum capacity of the biodigester per day) and mixed with tap water, adjusting to achieve two OL levels: OL5% and OL15%, with a ratio of 1:9 and 1:5, respectively. The OL was then decanted into the biodigester's influent pit.

Sampled Byproducts

Biogas

The biogas is conducted from the biodigester's dome through reinforced PVC piping. At a distance of 3 meters from this point, there is a valve on the piping for obtaining a biogas sample (1 cubic decimeter).

Wastewater

Directly from the outlet pipe of the biodigester at the discharge tank and in each of the lagoons in the lagoon system, a sample of the effluent was collected in a one-liter capacity container in its liquid physical form (liquid effluent). This sample was then transferred to Imhoff sedimentation cones to determine the Total Settled Solids (TSS) of the solid fraction, and the liquid fraction was stored in 1-liter capacity bottles at a temperature of 4 °C for subsequent analysis. (Figure 1).

Sample Analysis

Biogas: Using a LANTEC biogas measurement device, the percentage of methane (CH_4) and carbon dioxide (CO_2) present in the biogas was determined.

Wastewater: Chemical Oxygen Demand (COD) and the macronutrients Nitrogen (Total N) and Phosphorus (Total P) were determined for the liquid fraction of the effluent (Table 1).

Statistical Method: Two fixed levels of Organic Load (OL) were evaluated: OL5% and OL15%, over 4 periods of 40 days each, with 10 days of adaptation at each OL level. Sampling was conducted weekly in triplicate, averaging the biogas measurements per week. For the liquid effluent, samples were collected as the stabilization ponds were filled.

The obtained data were analyzed using differential statistics (biogas) and only descriptive statistics (wastewater), with the *R* 4.3.3 software, utilizing the following commands and packages: *fligner.test*, *shapiro.test*, *aggregate*, and *dplyr*. The *P*-value was calculated using a one-way ANOVA when the data came from normal and homogeneous distributions.

RESULTS AND DISCUSSION

Facilities and Organic Loads

The source of the organic waste was determined to be from gestating sows with an average weight of 220 kg, excreting an average of 1.8 kg of solid waste. According to daily manual cleaning, each sow consumed 7 liters of water per day. Chao *et al.* (2012) conducted a study on growing pigs (average weight 60 kg) and determined a water usage of 25.5 L per animal for cleaning and treating waste in pens, concluding that the water consumption is high and increases the cost of waste treatment. In this regard, it is suggested that manual cleaning methods could reduce water usage per animal, and consequently, reduce the organic load entering the biodigester, leading to lower costs for the post-treatment and reuse of this water.

Regarding the Organic Load (OL), various data provided by authors to determine the diluent proportions of manure: water and to establish Hydraulic Retention Times (HRT) have been reported (Cubillos-Sierra *et al.*, 2018), to evaluate the efficiency of organic matter removal in anaerobic biodigesters (Alonso-Estrada *et al.*, 2014). In this study, two Organic Loads were determined: a minimum of 5% (ratio 1:9) and a maximum of 15% (ratio 1:5) from the gestation area, considering a Dry Matter (DM) percentage of 30% in the manure

Table 1. Determination of physical-chemical parameters and nutritional concentration of the liquid effluent from a biodigester.

Parameter	Unit	Method
Environmental Temperature	°C	Climate station
CH_4	%	LANDTEC
CO_2	%	LANDTEC
pH y EC	μ S/cm	Potentiometer
COD	mg/L	HACH 8000
N-Total	mg/L	HACH 10072
F-Total	mg/L	HACH 10127
TSS	ml/L	Sedimentación Imhoff

Where: CH_4=methane; CO_2=carbon dioxide; EC=Electrical Conductivity; COD=Chemical Oxygen Demand; Total-N=Total Nitrogen; Total-P=Total Phosphorus; TSS=Total Settled Solids.

(Riascos-Vallejos *et al.*, 2018). Water was added to achieve the established levels for the study, defining the Hydraulic Retention Time as 30 days. The maximum recommended for optimal biodigester operation is 15% organic load, which makes maintenance intervals longer and ensures that with minimum and maximum OL, the effluent is more accessible to treat and meets the official standards established by NOM-001-SEMARNAT-2021. It is worth mentioning that with this percentage of Organic Load (OL), efficient biogas production and proportional quality parameters of methane relative to other components are ensured.

Byproducts

Biogas

Biogas is considered a primary byproduct, and its quality was determined in percentage terms (Table 2). Based on the OL levels, the quality and ratio of CH_4 and CO_2 were not affected in this study and, according to Sepúlveda *et al.* (2020), both OL levels fall within the acceptable methane range (40-70% CH-).

According to the treatments established in this study, an important factor that combines the quality of CH_4 with the secondary treatment of the water to be treated is the Total Settled Solids (TSS) (Figure 2). On one hand, the goal was to produce CH_4, and on the other hand, to achieve the treatment of the wastewater derived from the biodigester in the subsequent lagoons of the lagoon system.

In our case, the percentage of removal from liquid influent to liquid effluent was 66% for OL5% and 41% for OL15%, with a higher removal rate at the lower OL ($p>0.05$). This is because, inside the fermentation chamber, methanogenic bacteria consume more organic matter for their development and growth. These values are consistent with those

Table 2. Percentage of methane and carbon dioxide by organic load level.

Organic Charge level	Gas type	
	CH_4 %	CO_2 %
5%	$59.89^{a}\pm2.35$	$35.54^{b}\pm2.59$
15%	$60.25^{a}\pm6.9$	$34.92^{b}\pm6.51$

CH_4=methane; CO_2%=carbon dioxide; SD=standard deviation; Identical letters in each column indicate a p-value >0.05.

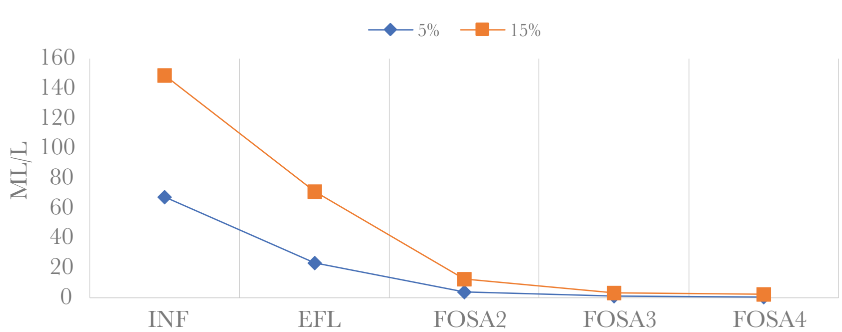

Figure 2. Total Settled Solids by Organic Load Level (5% and 15%).

described by Chibás *et al.* (2017), who reported a 57% removal of Total Settled Solids (TSS) in pig manure samples at the inlet and outlet of biodigesters. Additionally, Trejo-Lizama *et al.* (2014) noted that 50% of the sampled biodigesters in the Yucatán region achieved reference values for organic matter removal. However, they require additional treatments to continue the wastewater treatment process, as was implemented in this study with the addition of three more treatment ponds.

Highlighting the importance of a lagoon system as a complementary treatment, some physical-chemical parameters and macronutrients were determined (Table 4). In both OL levels, the pH value is below neutrality, which is expected because the excretions are acidic in influent (INF). In the case of the effluent (EFL), the pH in the anaerobic fermentation chamber remains in this slightly acidic range. Additionally, the continuous feeding flow of the biodigester carries water from the mixture during internal agitation.

In the subsequent ponds, the pH exceeds neutrality as the Total Settled Solids (TSS) decrease and as Hydraulic Retention Time (HRT) progresses in each pond. These values align with the range (pH 6.0 to 7.8) reported by Cano *et al.* (2016), who worked with liquid pig and cattle effluents over a period of 45 days.

García (2012) defines Electrical Conductivity (EC) as the capacity of water to conduct an electrical current. Pérez González & Mata Varela (2016), working with 6 scale biodigesters fed with pig manure, found an average EC of 3905.3 μ/cm at the outlet of these digesters. In a study of raw and treated wastewater, the average EC was 1763 and 1833 μ/cm, respectively, which is considered high concentration (García-Carrillo *et al.*, 2020). In relation to this study, for both OL levels, the EC in the influent (raw) is similar to the previous study; however, for the effluent (treated), the concentration doubles, as well as in the subsequent ponds. The increase in this parameter from influent to effluent is due to the concentration of dissolved salts in the fermentation chamber, with no consumption of salts by microorganisms despite the organic matter degradation. Furthermore, concentrations rise in the subsequent ponds, exceeding the maximum permissible limits of the (NOM-001-SEMARNAT-2021, which establishes permissible contaminant limits for wastewater discharges into national bodies of water, 2021), which is $<$1000 μ/cm.

Chemical Oxygen Demand (COD) is one of the main parameters for determining water quality. It measures the amount of oxygen required to oxidize the organic matter susceptible to oxidation in a liquid sample and establishes a level of contamination (Rosabal-Carbonell *et al.*, 2012). According to Garzón-Zuñiga & Buelna G. (2014), they measured the performance of an anaerobic digester and two series stabilization lagoons with high concentrations of OL from the farrowing area, with a Hydraulic Retention Time (HRT) $>$60 days, at a farm with 5600 sows. These authors achieved a COD removal of 81.6% at the biodigester outlet. Lansing *et al.* (2008), working with a small-scale biodigester where the excretions came from 12 pigs with an HRT of 44 days, found a COD removal of 87% when measuring the influent and effluent of the biodigester. In this study, by establishing the OL and HRT and measuring the importance of the lagoon treatment train along with the biodigester, we found a removal greater than $>$50% from the influent to the fourth stabilization pond. At the effluent outlet in both OL levels, a higher amount of oxidant is required because the influent, upon contact in the fermentation chamber, contains undegraded organic matter, indicating persistent levels of contamination. As the wastewater progresses through the stabilization ponds, this organic matter decreases. When comparing the two previous studies, it is important to note that the amounts of OL and HRT are different.

Regarding the nutrients present in the liquid effluents subjected to a digestion process (Table 3), Total Nitrogen (TN) and Total Phosphorus (TP) were prominent. In a pig farm, producing an average of 10.2 kg of solid waste, the water used to feed a biodigester originated from the washing process of the pens, at a ratio of 1:4 (manure: water) with an HRT of 43 days. Peñafiel *et al.* (2021) obtained 230±18 *vs.* 373±27 mg/L of TN and 53±5.7 *vs.* 290±21 mg/L of TP, from influent to effluent of the biodigester, respectively. Comparing with this study, it was observed that, very likely due to the similarity of OL and shorter HRT, the two macronutrients increased, indicating that during the anaerobic fermentation process, most of the nutrients in the liquid are retained (Martínez-Hernández & Francesena-López, 2018).

In another study, working with bovine waste, Cabos Sánchez *et al.* (2019) observed that the Total Nitrogen (TN) initially decreased and then increased over time in the liquid effluent measurements. In the case of Total Phosphorus (TP), it decreased over time. The author concluded that these variations depend on the type and amount of Organic Load (OL) used to achieve the concentrations. In this study, Total Nitrogen (TN) was retained as it passed through the ponds, while Total Phosphorus (TP) decreased. According to the

Table 3. Nutrients in influent, liquid effluent, and pig wastewater treatment ponds by Organic Load level.

Simple origin	Organic Charge level			
	5%		15%	
	NT mg/L	FT mg/L	NT mg/L	FT mg/L
INF	128.33±145.9	173.16±184.4	111.90±123.4	130.87±171.7
EFL	436.93±142.1	249.44±210.0	554.62±345.4	309.29±319.6
Fossa 2	348.09±116.8	84.87±52.4	464.36±134.0	103.56±191.5
Fossa 3	267.91±169.4	37.96±24.7	365.25±146.3	40.51±35.1
Fossa 4	217.32±145.86	37.90±24.1	342.49±82.2	17.59±18.2

Where: INF=Influent; EFL=Liquid Effluent; TN=Total Nitrogen; TP=Total Phosphorus; mean ± standard deviation.

Table 4. Physicochemical parameters of influent, liquid effluent and pig wastewater treatment fossas by organic load level.

Sample origin	Organic load level							
	5%				15%			
	TSS ml/L	pH	EC μs/cm	COD mg/L	TSS ml/L	pH	EC μs/cm	COD mg/L
INF	67.45±8.21	6.1±0.17	2225.0±1488.5	738.72±1003.6	81.38±64.62	6.3±0.18	1842.5±757.4	1807.7±3452.8
EFL	23.27±29.06	6.7±0.31	3503.2±1085.9	1444.2±844.2	48.02±76.7	6.6±0.25	4705.2±1247.7	2522.5±2745.6
Fossa 2	3.98±7.44	7.2±0.18	3669.42±514.6	677.0±449.1	8.51±7.87	7.2±0.16	2942.5±927.4	1057.7±484.6
Fossa 3	1.26±1.19	7.4±0.08	2983.3±853.6	600.2±471.7	2.02±1.99	7.4±0.15	3366.7±1258.5	708.3±335.3
Fossa 4	0.51±0.24	7.6±0.16	3228.0±659.9	690.6±348.4	1.89±3.13	7.6±0.11	3546.5±1363.5	409.1±128.9

Where: INF=Influent; EFL=Liquid effluent; TSS=Total Settled Solids, EC=Electrical Conductivity; COD=Chemical Oxygen Demand.

resulting concentrations, the Organic Load (OL) levels should be considered, and the post-treatment with stabilization ponds having Hydraulic Retention Time (HRT) should be included. The filling times of the biodigester should be standardized, and the type of organic matter intended for the fermentation chamber should be established to determine compliance with the maximum permissible limits set by NOM-001-SEMARNAT-2021.

CONCLUSIONS

Based on the obtained results, the physicochemical and nutritional parameters of liquid piggery effluent derived from a biodigester complemented by a lagoon system, it is essential to determine a maximum and minimum Organic Load (OL) to ensure a good percentage of methane. Since the liquid effluents do not comply with the established Official Mexican Standards (NOMs), they continue to be a problem for producers due to lack of training, resources, and environmental awareness. Therefore, the implementation of additional systems to the biodigester for wastewater treatment (known as complementary treatments) is necessary. Biodigesters should be integrated into a waste management system so that their products serve as a basis for implementing another process or system.

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In 2009, the Livestock Production Module was integrated into the Centro-Altos de Jalisco Experimental Field, with a focus on the Sustainable Integral Production System (SIPIS).

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Colegio de Postgraduados

Relationship between feeding protocols and their cost with body development of lactating calves in family milk-production units

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ABSTRACT

Objective: Characterize the feeding protocols and feeding costs, as well as their relationship with the body development of lactating calves until weaning in family milk-production units (FMPU).

Methodology: A prospective observational cohort study was carried out. The feeding protocol, and its cost were recorded, in addition, the daily gains in weight (DWG) and height (DHG) between birth and weaning of 193 calves (n=12 FMPU). The information was subjected to descriptive statistics and analysis of variance.

Results: In some FMPU, up to 5 feeding protocols were used, and calves with a range of 49 to 138 days until weaning. The predominant feeding consisted of milk replacer plus starter concentrate (28.5%). The costs of the feeding protocols (1162 to 2395 pesos), as well as the DWG (0.346 to 0.721 Kg/day) and DHG (0.114 to 0.216 Cm/day), were statistically different between FMPU ($P<0.01$). The most expensive feeding protocol had a DWG of 0.555 kg/day and a DHG of 0.161 cm/day.

Limitations of study/implications: The nutrient contributions of the feeding protocols were not determined, consequently, whether they covered the calves' nutritional requirements.

Conclusions: In FMPU, there is a lack of standardization in feeding protocols and a high variation in their costs, as well as in the duration to weaning. The body development of the calves is suboptimal, influenced by the feeding protocol, where the highest cost is not reflected in the best body development rate.

Keywords: Economic cost, body development, Holstein calves.

INTRODUCTION

The replacement heifer rearing process represents one of the most significant production costs in dairy herds. Typically, the investment begins to be recouped when the heifers reach

their first lactation, although full recovery may not occur until even the sixth lactation (Boulton *et al.*, 2017). Therefore, it is essential to direct efforts to ensure that replacements achieve an adequate body development rate at the lowest possible economic cost, enabling them to express their full productive potential throughout their adult life (Akins, 2017).

In production units that use the Holstein breed, performance indicators have been established to assess whether replacement rearing objectives are being met. It is accepted that heifers should conceive between 13 and 15 months of age, reaching 55% of their mature body weight, so that they can calve for the first time between 22 and 25 months of age, reaching 85% of their mature body weight (Akins, 2017; DCHA, 2016). In small-scale family dairy farms, which contribute approximately 30% of the national milk production, it has been reported that replacements receive their first service at an average age of 20 months and have their first calving at an average age of 29 months (Espinosa-Martínez *et al.*, 2012). These values indicate that the rearing objectives are not being met.

During the heifer rearing process, the lactation period coincides with the time when they are most susceptible to issues affecting their viability, body development, and consequently, their future performance (Bazeley *et al.*, 2016; Urie *et al.*, 2018a). Additionally, this period is costly due to the prices of the feed required for calves (Heinrichs *et al.*, 2013) and is a critical period to promote adequate ruminal development, allowing them to subsequently utilize lower-cost feeds such as forages (Akins, 2016). However, the body development of calves, as well as production costs during lactation, will largely depend on the feeding conditions to which they are exposed (Svensson and Hultgren, 2008; Heinrichs *et al.*, 2013; Boulton *et al.*, 2017). In this regard, it is observed that in family dairy production systems, feeding management is often inadequate and calves body development rates are commonly suboptimal from birth until weaning, negatively affecting the achievement of rearing objectives (Gutiérrez-Morales, 2014; Villaseñor-González *et al.*, 2022).

In this regard, the hypothesis proposed is that the limited body development of lactating calves may be influenced by the feeding protocols used. However, little has been explored in this respect within the family production system in Mexico. Therefore, the objective of the present study was to characterize the feeding protocols and costs used, as well as their relationship with the body development of lactating calves up to weaning in family milk-production units.

MATERIALS AND METHODS

Location of the Experimental Area

The study was conducted in the municipalities of Tepatitlán de Morelos, San Ignacio Cerro Gordo, and Valle de Guadalupe, which are part of the Los Altos dairy basin in the State of Jalisco, Mexico. These municipalities are located between 20° N and 102° W at an altitude ranging from 1680 to 2100 meters above sea level. The climate of the region is temperate sub-humid with an average temperature of 19.0 °C and an average annual precipitation of 753.0 mm between the months of June and September (CEAJ, 2023).

Experimental Units and Data Collection

A prospective cohort observational study was conducted, including 193 Holstein calves from 12 production units with characteristics of the family dairy production system (Montiel-Olguín *et al.*, 2019). Weekly visits were made to each cooperating production unit to record economic aspects and body development of the calves from birth until weaning.

Feeding Protocols and Costs

The type, quantity, and cost of each feed offered weekly to each calf were determined for the different feeding protocols used by each production unit. This was done based on records generated daily by the personnel in charge of the calves and the research team at each production unit, using field sheets. Additionally, during each visit by the research team, the consistency of the recorded data was verified, and direct evaluations of the amount of feed supplied (both liquid and solid) were conducted.

The quantity in liters of liquid feed (colostrum, whole milk, and milk replacer) offered was obtained from measurements taken in the containers (buckets) used to supply this type of feed. On the other hand, the quantity of solid feed (concentrate and forage) offered was estimated through weight measurements of the portions provided. Subsequently, the costs per type of feed and per feeding protocol offered to each calf during lactation were calculated, considering the combination of liquid and solid feed.

Body Development

The body development of the calves was determined through daily weight gain (DWG) and daily height gain (DHG) from the period of birth to weaning. For this purpose, each calf's birth weight (BW), birth height (BH), weaning weight (WW), and weaning height (WH) were recorded. Body weight was directly estimated through the thoracic perimeter, using measuring tapes specifically graduated for use with Holstein calves (Dairy Calf Tape, Coburn Co., Whitwater WI) and based on the methodology established by Heinrichs *et al.* (1992).

To estimate height, a stadiometer was used to measure the distance from the ground to the withers of the calves. To obtain the daily weight gain (DWG), the following formula was used:

$$DWG = (WW - BW) / \textit{Days in lactation}\ (DL)$$

and to obtain the daily height gain (DHG), the following formula was used:

$$DHG = (WH - BH) / DL$$

This is based on the formula described by Villaseñor *et al.* (2022).

Statistical Analysis

Data on the type of liquid feed, type of solid feed, and the final feeding protocol offered to each calf were subjected to descriptive frequency statistics analysis. The variables of final feeding cost, DWG, and DHG were subjected to variance analysis, with the production unit included as an independent variable. In each statistical model to evaluate the final feeding cost, DWG, and DHG, DL, BW, and BH were included as covariates, respectively. As an example of each model:

$$Y_{ij} = \mu + \alpha_i + \beta\left(X_{ij} - \bar{X}\right) + \varepsilon_{ij}$$

where: Y_{ij}=*ij*-th observation of the response variable Y; μ=overall mean of the response variable; α_i=effect of the *i*-th treatment; β=regression coefficient between X and Y; X= *ij*-th observation of the covariate X; $\bar{X}$ =overall mean of the covariate X; ε_{ij}=random error of the *ij*-th observation.

For all analyses, version 9.3 of the SAS statistical software package was used, and in the variance analyses, the Generalized Linear Models procedure was employed (SAS, 2011). Probability values ≤0.05 and >0.05 up to ≤0.1 were considered significant or indicative of a statistical trend, respectively.

RESULTS AND DISCUSSION

Overall, the results support the hypothesis that the deficient body development observed in the calves is influenced by the feeding protocols implemented. In the present study, it was possible to identify the frequencies of liquid and solid feeding used from birth to weaning of calves in family milk-production units. Information was also obtained that allowed for the analysis of the relationship between feeding costs, lactation duration, and the body development of the heifers.

In Table 1, milk replacer was the most used type of liquid feed (46.1%), followed by whole milk (36.8%), and to a lesser extent, a combination of milk replacer and whole milk (17.1%). This pattern regarding the use of milk replacer is consistent with previous studies (Urie *et al.*, 2018a), where producers attempt to reduce costs by including milk replacer, although in many cases it negatively affects calf growth (Lee *et al.*, 2009). Regarding solid feed, starter concentrate was the most used type (49.2%), followed by a combination of starter concentrate and corn stover with grain (29.0%). However, it was observed that 21.8% of the calves received one of eight different types of solid feed (Table 1). This indicates that, while most production units use a starter concentrate designed for calves, low nutritional value forages such as corn stover without grain, which has a low protein content (NASEM, 2021), and concentrates designed for adult cattle in production are also used.

Regarding final feed, the use of milk replacer plus starter concentrate predominated (28.5%). Additionally, a similar percentage (around 14.0%) was found for the use of milk replacer plus starter concentrate plus corn stover with grain, whole milk plus starter concentrates, and whole milk plus starter concentrate plus corn stover with grain. In Table 2, it can be observed that 50% of the production units employed different final feeding protocols during the study period, with some cases using up to five types. These results indicate that the feeding process for calves is not technically designed or standardized in half of the family production units. This feeding management contrasts with what

Table 1. Types of feed and their usage percentages in the calves.

Type of feed	Percentage (Frequency)
Liquid feed	
Whole milk	36.8 (71/193)
Milk replacer	46.1 (89/193)
Whole milk+Milk replacer	17.1 (33/193)
Solid feed	
Starter concentrate	49.2 (95/193)
Starter concentrate+other concentrate*	2.1 (4/193)
Starter concentrate+CSWG	29.0 (56/193)
other concentrate+CSWG	7.3 (14/193)
Starter concentrate+alfalfa hay	6.2 (12/193)
Starter concentrate+other concentrate*+CSWG	2.1 (4/193)
Starter concentrate+CSiWG	0.5 (1/193)
Starter concentrate+other concentrate*+CSiWG	1.6 (3/193)
Starter concentrate+other concentrate*+alfalfa hay+CSiWG	1.0 (2/193)
Starter concentrate+CSWoG	1.0 (2/193)
Final Feed[&]	
1.- MR+starter concentrate	28.5 (55/193)
2.- MR+starter concentrate+CSWG	14.5 (28/193)
3.- WM+MR+starter concentrate+CSWoG	1.0 (2/193)
4.- WM+starter concentrate	14.0 (27/193)
5.- WM+starter concentrate+CSWG	14.0 (27/193)
6.- WM+MR+starter concentrate	7.3 (14/193)
7.- WM+MR+other concentrate*+CSWG	7.3 (14/193)
8.- MR+starter concentrate+alfalfa hay	4.7 (9/193)
9.- WM+starter concentrate+other concentrate*+CSWG	2.0 (4/193)
10.- WM+starter concentrate+CSiWG	0.5 (1/193)
11.- WM+starter concentrate+other concentrate*+CSiWG	1.6 (3/193)
12.- WM+starter concentrate+other concentrate*	2.1 (4/193)
13.- WM+starter concentrate+other concentrate*+alfalfa hay+CSiWG	1.0 (2/193)
14.- WM+starter concentrate+alfalfa hay	1.5 (3/193)

*Concentrate not specific for lactating calves. [&]Combination of liquid and solid feed provided. MR=Milk replacer; WM=Whole milk; CSWG=Corn stover with grain; CSWoG=Corn stover without grain; CSiWG=Corn silage with grain.

occurs in intensive production units, where it is recommended to follow a standardized feeding protocol for calves (Akins, 2016). For example, a survey conducted in U.S. dairies showed that pre-weaning feeding practices exhibit high similarity (Urie *et al.*, 2018a). Conventionally, it is recommended that calves consume starter concentrate containing 18 to 22% protein and high levels of fermentable carbohydrates (up to more than 35%), as well as high-quality forages such as alfalfa hay from the first weeks of life. This allows them to stimulate ruminal development and better adapt to changes in feeding (Akins, 2016; Machado and Ballou, 2022).

Additionally, it was observed that the initiation of starter concentrates and forage feeding for the calves was highly variable, with some dairies starting from the first week and most beginning after the third or fifth week of age. This management also contrasts with the conventional recommendation for Holstein calves, which suggests gradually integrating solid feed in increasing amounts throughout the lactation period (Machado and Ballou, 2022). Early provision of forage (from the first week of age) could compromise nutrient digestibility and calves' growth, compared to early feeding without hay or starting hay consumption from the second week (Xiao *et al.*, 2023). The results reflect the diversity in producers' criteria and the possible lack of technical guidance for managing feeding protocols, highlighting the need for improvement in this process. However, it is possible that these decisions are influenced by the availability and cost of different feeds at specific times throughout the year.

When analyzing the duration of the lactation period by production unit and type of feed, it was identified that calves remain in lactation for an average range of between 49 and 138 days, with variable times within the same production unit (Table 2). However, most production units had a lactation period between 60 and 90 days. The results regarding

Table 2. Types of final feed in each production unit and average days in lactation for each group of calves.

Production unit	Final feed (n)	Days at weaning
1	2.- MR+starter concentrate+CSWG (1/23)	64.0
1	4.- WM+starter concentrate (10/23)	68.0
1	5.- WM+starter concentrate+CSWG (9/23)	72.0
1	6.- WM+MR+starter concentrate (3/23)	70.3
2	1.- MR+starter concentrate (3/16)	65.7
2	3.- WM+MR+starter concentrate+CSWoG (2/16)	65.0
2	6.- WM+MR+starter concentrate (11/16)	62.3
3	4.- WM+starter concentrate (4/13)	99.5
3	10.- WM+starter concentrate+CSiWG (1/13)	84.0
3	11.- WM+starter concentrate+other concentrate*+CSiWG (3/13)	100.0
3	13.- WM+starter concentrate+other concentrate*+alfalfa hay+CSiWG (2/13)	138.0
3	14.- WM+starter concentrate+alfalfa hay (3/13)	78.3
4	2.- MR+starter concentrate+CSWG (27/27)	77.0
5	7.- WM+MR+other concentrate *+CSWG (14/14)	94.7
6	1.- MR+starter concentrate (17/17)	49.4
7	1.- MR+starter concentrate (14/14)	62.3
8	8.- MR+starter concentrate+alfalfa hay (4/4)	84.3
9	1.- MR+starter concentrate (7/12)	60.3
9	8.- MR+starter concentrate+alfalfa hay (5/12)	61.4
10	4.- WM+starter concentrate (13/29)	63.4
10	5.- WM+starter concentrate+CSWG (16/29)	66.9
11	5.- WM+starter concentrate+CSWG (2/10)	90.5
11	9.- WM+starter concentrate+other concentrate*+CSWG (4/10)	88.5
11	12.- WM+starter concentrate+other concentrate* (4/10)	89.5
12	1.- MR+starter concentrate (14/14)	62.3

*Concentrate not specific for lactating calves.
MR=Milk replacer; WM=Whole milk; CSWG=Corn stover with grain; CSWoG=Corn stover without grain; CSiWG=Corn silage with grain.

the duration of lactation observed in the present study are similar to those described in previous studies within this production system (Urie *et al.*, 2016a; Villaseñor-González *et al.*, 2022). It has been noted that lactation constitutes the stage of highest daily economic expenditure in calf rearing due to the costs of liquid feed (Heinrichs *et al.*, 2013). Indeed, in the present study, it was observed that liquid feed represented, on average, 80.2% (see Table 3) of the final feed, with a cost ranging from $589.5 to $3,776.6 Mexican pesos.

As shown in Table 3, another factor contributing to the increase in final feed cost was the duration of lactation, as weaning at 41 days cost $679.9 and $4,908.3 at 155 days. In contrast, other authors recommend that Holstein calves should be weaned at 60 days and consuming at least 700 g/day of concentrate for at least three consecutive days (Eckert *et al.*, 2015; Urie *et al.*, 2018b). This management practice helps to reduce costs during lactation and prevents potential growth setbacks in calves around weaning (Eckert *et al.*, 2015; Urie *et al.*, 2018b).

Significant statistical differences were found between production units ($P<0.01$) for both DGW and DHG, as well as for the cost of feeding (Table 4). Regarding calves' development, average development rates were below 600 g/day in 10 of the 12 evaluated production units, and in the other two units, the rates did not exceed 725 g/day. Similar results regarding DGW have been observed in previous studies (Gutiérrez-Morales, 2014; Villaseñor-González *et al.*, 2022), highlighting the need to pay more attention to the calf rearing process. It has been found that growth problems in the early stages of calf life can negatively affect their future productive and reproductive performance (Bazeley *et al.*, 2016; Van de Stroet *et al.*, 2016; Vam Eetvelde and Opsomer, 2017). Regarding DHG, similar results to those in the present study have also been described previously (Villaseñor-González *et al.*, 2022).

There was wide variation in feeding costs among the evaluated production units. It was observed that calves receiving the most expensive feeding protocol did not necessarily have the best weight or height gains (production units 3, 6, 9, and 10). On the other hand, calves that showed better body development received a feeding protocol with intermediate costs (production units 4 and 11). For example, calves in production unit 3 received an average of 0.84 kg day^{-1} of solid feed (>90% concentrate) and 3.6 liters/day of liquid feed (whole milk); meanwhile, calves in production unit 11 received 0.99 kg of solid feed (>90%

Table 3. Descriptive statistics of the cost (in Mexican pesos) of solid feed, liquid feed, and final feed for the total calves.

Descriptive Statistic	Solid Feed n=193	Liquid Feed n=193	Final Feed[&] n=193	Days at Weaning n=193
Mean	363.2	1470.1	1833.4	71.2
Minimum	40.1	589.5	679.9	41
First quartile	168.8	1250.0	1505.1	61
Second quartile	304.6	1438.4	1823.7	67
Third quartile	527.3	1655.6	2073.7	78
Maximum	1131.7	3776.6	4908.3	155

[&]Combination of liquid and solid feed provided.

Table 4. Effect of the production unit on the cost (in Mexican pesos) of feeding, daily weight gain (DGW), and daily height gain (DHG) of the calves.

Production Unit (n)	Feed Cost**	DGW Kg**	DHG Cm**
1 (23)	1955.5 ± 45.4^{c}	0.584 ± 0.03^{b}	0.187 ± 0.01^{bcd}
2 (16)	1513.8 ± 56.5^{e}	0.473 ± 0.03^{cdef}	0.166 ± 0.01^{cdef}
3 (13)	2395.8 ± 81.8^{a}	0.555 ± 0.04^{bc}	0.161 ± 0.01^{cdef}
4 (27)	1799.3 ± 43.3^{d}	0.721 ± 0.02^{a}	0.216 ± 0.01^{a}
5 (14)	1268.5 ± 74.0^{f}	0.511 ± 0.03^{bcd}	0.153 ± 0.01^{def}
6 (17)	2183.2 ± 67.9^{ab}	0.450 ± 0.03^{def}	0.156 ± 0.01^{def}
7 (14)	1825.0 ± 60.6^{d}	0.414 ± 0.03^{ef}	0.143 ± 0.01^{ef}
8 (4)	1172.3 ± 111.5^{f}	0.346 ± 0.06^{f}	0.114 ± 0.03^{f}
9 (12)	2070.2 ± 66.0^{bc}	0.519 ± 0.04^{bcd}	0.148 ± 0.02^{ef}
10 (29)	2099.3 ± 41.9^{b}	0.470 ± 0.02^{def}	0.170 ± 0.01^{cde}
11 (10)	1781.8 ± 77.2^{d}	0.705 ± 0.04^{a}	0.215 ± 0.02^{ab}
12 (14)	1162.0 ± 60.6^{f}	0.490 ± 0.03^{cde}	0.198 ± 0.01^{abc}

**$P<0.01$ level for the effect of the production unit; data presented as means ± standard error. [abcdef] Different letters among means within each response variable indicate significant statistical difference.

concentrate) and 3.4 L of liquid feed (whole milk). Additionally, not all types of low-cost feed resulted in the lowest body development in calves.

In the present study, the amount of liquid and solid feed provided was quantified; however, individual consumption and the nutritional contribution of the final feed could not be determined, which limits the ability to explain the results from this important aspect of calf' body development. These observations suggest an opportunity to improve nutritional management and obtain appropriate growth indicators at a lower cost. Other factors, such as housing conditions (Gutiérrez-Morales, 2014), maternal characteristics during the peripartum period such as body condition or having received a vaccination schedule (García-González, 2016), and colostrum consumption at birth (Villaseñor-González *et al.*, 2022), could also be associated with the observed body development. However, these variables were not recorded in this study.

CONCLUSIONS

In family milk-production units, there is a lack of standardization in feeding protocols. Although most units use feeds designed for lactating calves, some still utilize feeds of low nutritional value that are not suitable for this developmental stage. The increase in feeding costs is influenced by the level of liquid feed used and the duration of the calf's lactation. The body development of calves in most production units is suboptimal and is influenced by the implemented feeding protocol. Higher feeding costs do not necessarily result in better body development rates for calves, highlighting the need for actions aimed at improving feeding practices and achieving adequate calf's development at a lower cost.

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Reproductive Response of Hair Sheep to Short or Long Duration Estrus Synchronization Protocols

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ABSTRACT

Objective: Evaluate the reproductive response of hair ewes treated with estrus synchronization protocols that included a progesterone-releasing intravaginal device (CIDR) for a period of 7 or 12 days.
Methodology: During the end of winter, ewes were randomly allotted to one of two estrus synchronization protocols: 1) Short-term protocol (SD), ewes (n=24) received a CIDR on Day 1 and removed on Day 7, and received 400 IU of equine chorionic gonadotropin (eCG) and 0.125 mg of cloprostenol; 2) Long-term protocol (LD), ewes (n=24) received a CIDR on Day 1, retired on Day 12 and received 400 IU eCG. Ewes showing estrous behavior were bred with a mature ram.
Results: There were no differences ($P>0.05$) in the estrus response in both groups of ewes (100 *vs.* 95.8% for SD and LD, respectively), the pregnancy rate (87.5 *vs.* 87.5%), prolificacy (1.95 ± 0.1 *vs.* 2.2 ± 0.1 lambs/ewe), hours between CIDR removing and the beginning of estrus (34.4 ± 8.1 *vs.* 34.8 ± 9.2 h), and estrus duration (39.7 ± 2.9 *vs.* 39.9 ± 4.7 h). At CIDR removal, ewes in the LD group showed a greater diameter ($P<0.05$) for the largest follicle in both ovaries (5.42 ± 0.3 and 5.42 ± 0.4 mm for the follicle in the left and right ovaries, respectively) *vs.* SD ewes (4.25 ± 0.1 and 4.29 ± 0.1 mm).
Limitations of study: Results observed in the follicle diameter could be due to low circulating progesterone levels in the LP group, which must be confirmed in future studies.
Conclusions: The short protocol for estrus synchronization can be used in hair ewes under temperate conditions, without affecting their reproductive response.

Keywords: CIDR, Reproduction, Pregnancy rate, Prolificacy.

Image by Alejandro Sánchez

INTRODUCTION

Estrus synchronization is a widely used technology in reproductive programs for ruminant species (Habeeb *et al.*, 2021; Hameed *et al.*, 2021). In the literature, various protocols have been published for sheep, such as those considering the use of prostaglandins or their analogs (Alavez-Ramírez *et al.*, 2014; De Carvalho *et al.*, 2018), as well as those using progesterone or progestogens, which can be used alone or combined with other hormones like equine chorionic gonadotropin (eCG) and/or prostaglandins, with a total protocol duration of up to more than 12 days (Oliveira *et al.*, 2016; Rosasco *et al.*, 2019; Santos-Jimenez *et al.*, 2022).

In this context, there is insufficient information on follicular dynamics that supports the use of treatments with different durations, especially in short protocols. This is because after five or six days of inserting intravaginal progesterone-releasing devices (Arroyo-Ledezma *et al.*, 2013; Cox *et al.*, 2012) or progesterone-impregnated sponges (Alavez-Ramirez *et al.*, 2014), the blood concentration of this hormone decreases. It has been suggested not to maintain the devices for more than seven days, which necessarily requires the application of prostaglandins upon removing the source of exogenous progesterone (Arroyo-Ledezma *et al.*, 2013).

Estrus synchronization protocols based on progestogens for only seven days have been previously described (Bruno-Galarraga *et al.*, 2021; Santos-Jimenez *et al.*, 2022), although they are less known among technicians and producers. Their use not only allows for a reduction in cost but also facilitates the development of new protocols involving minimal exposure to exogenous hormones. Although these protocols are effective, the reproductive response to them has not been clearly evaluated in hair sheep outside of warm, tropical, and subtropical regions, where they have their natural habitat (Aguilar-Martínez *et al.*, 2017). Additionally, these sheep exhibit reproductive characteristics different from other breeds, such as a shorter anestrous period (Arroyo, 2011).

The results of estrus synchronization protocols can be influenced by variables such as breed, duration of exposure to progestogens, additional hormones used (prostaglandins, equine chorionic gonadotropin), environmental factors, etc. (Alavez-Ramirez *et al.*, 2014; De *et al.*, 2020; González-Reyna *et al.*, 2014; Texeira *et al.*, 2016). Therefore, the objective was to evaluate the reproductive response of hair sheep to short and long duration estrus synchronization protocols, based on controlled-release progesterone devices (CIDR), under a temperate climate. The hypothesis was that the use of a short-duration estrus synchronization protocol could elicit at least a similar reproductive response in sheep compared to a long-duration protocol.

MATERIALS AND METHODS

Animals and Geographical Location

Adult hair sheep (1 to 6 years old) of Pelibuey (n=39) and Blackbelly (n=9) breeds were used, with an average body weight of 48.9±1.5 kg. The study was conducted at the end of winter and the beginning of spring, with increasing daylight hours in the state of Querétaro, Mexico (20° 42’ N, 100° 01’ O). March is routinely considered the breeding season at the production unit. The climate in the study region is temperate, semi-arid, with an average annual temperature of 17.4 °C. The sheep were fed a diet (forage and concentrate) that met their nutritional requirements according to their physiological stage, as described by the NRC (2007), and always had free access to water.

Treatments

The sheep were randomly assigned to one of two estrus synchronization protocols, which differed in their duration of exposure to exogenous progesterone. In the short-duration protocol (SD; n=24), the sheep received an intravaginal device impregnated with 0.3 g of progesterone (CIDR®; Zoetis, NJ, USA) on day 1. On day 7, the CIDR was removed, and the sheep received an intramuscular dose of 400 IU of eCG (Folligon®; Intervet, USA) and 0.125 mg of Cloprostenol (Inducel®; Virbac, Carros, France) to remove any functional corpus luteum in the sheep (Arroyo-Ledezma *et al.*, 2013). In the long-duration protocol (LD; n=24), the sheep received the CIDR on day 1 and it was removed on day 12, in addition to receiving an intramuscular dose of 400 IU of eCG.

At the beginning of the study, the sheep were weighed, and their body condition was assessed according to Romero (2015), using a scale from 1 to 5, where 1 corresponds to a sheep in a state of emaciation and 5 corresponds to an obese sheep. Additionally, at the beginning and end of the synchronization treatment, both ovaries were scanned using transrectal ultrasonography, with an ultrasound machine (Aloka, model SSD500), equipped with a 5.0 MHz transrectal probe. All follicles equal to or larger than 3 mm in both ovaries were recorded.

Estrus detection was carried out twice a day (08:00 and 17:00 h) between 24 and 96 h after CIDR removal, using a mature ram. Ewes exhibiting signs of estrus (those that remained immobile) were naturally bred with one of three mature rams, each with a history of good fertility during previous breeding seasons, at a ratio not exceeding 1:10. The different durations of the treatments allowed the same rams to be used for both groups of ewes without changing the ratio.

Subsequently, the time elapsed between CIDR removal and the onset of estrus, as well as the duration of estrus, was recorded. Twenty-eight days after exposure to the rams, pregnancy diagnosis was performed using transrectal ultrasonography with a 5.0 MHz probe. Finally, at lambing, the number of lambs born per ewe (prolificacy) was recorded.

Statistical Analysis

Frequency data (estrus rate and conception rate) were analyzed using Fisher's exact test to determine differences between synchronization protocols. Body weight, body condition, time to exhibit estrus behavior, duration of estrus, diameter of the largest follicle, and number of follicles were analyzed using Student's t-test.

Body condition data, time to estrus presentation, and estrus duration were transformed using the natural logarithm option prior to analysis, while data on the diameter of the largest follicle, number of follicles, and prolificacy were transformed using the square root of (y+0.5). To facilitate understanding, all mean values and standard errors are presented as their original untransformed values. All analyses were conducted using the SAS statistical software package (SAS Institute Inc., Cary NC, USA).

RESULTS

One hundred percent of the devices remained within the ewes' vaginas during the treatment periods considered. Additionally, no abnormal vaginal discharges were observed after their removal.

Body Weight and Condition. At the beginning of the study, no differences (P>0.05) were observed in body weight and body condition score between the synchronization groups (Table 1).

Estrus and Conception. There were no differences between treatments (P>0.05) regarding estrus rate, conception rate, and prolificacy (Table 1). For ewes that exhibited estrus exclusively, the conception rate was 87.5% and 91.3% for the SD and LD groups (P>0.05). The average time elapsed from CIDR removal to the onset of estrus behavior was less than 35 h (P>0.05) for both synchronization protocols, and the duration of estrus lasted nearly 40 h (P>0.05).

The timing of estrus occurrence was similar for both treatment groups (Figure 1). Within 24 hours after CIDR removal, only 16.7% of the ewes had shown estrus in both groups;

Table 1. Reproductive Response of Hair Sheep to Short (7 d) or Long Duration (12 d) Estrus Synchronization Protocols.

Variable	Short protocol[1]	Long Protocol[2]	P value
n	24	24	-
Body weight, kg	49.4±2.2	48.5±2.0	NS
Body condition score	2.81±0.08	2.68±0.09	NS
Estrus rate, %	100	95.8	NS
Total conception rate, %[†]	87.5	87.5	NS
Onset of estrus[††], h	34.4±1.7	34.8±1.9	NS
Estrus duration, h	39.7±2.9	39.9±4.7	NS
Prolificacy	1.95±0.15	2.2±0.14	NS

[1] Insertion of CIDR on day 1, removal on day 7 + eCG and Cloprostenol; [2]Insertion of CIDR on day 1, removal on day 12 + eCG. [†] Pregnant ewes/ewes in the breeding group; [††] Time after CIDR removal. NS=Not significant (P>0.05). Results are expressed as mean ± standard error.

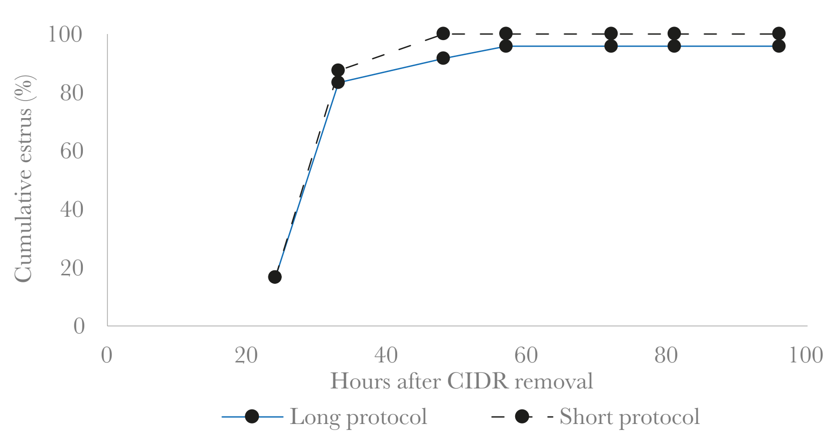

Figure 1. Cumulative Percentage of Estrus in Ewes with Short (CIDR for 7 days) or Long Duration (CIDR for 12 days) Estrus Synchronization Protocol.

however, by 33 hours, this percentage had increased dramatically (86.5%). Ewes that responded to the synchronization protocol did so fully by 57 hours after CIDR removal. The highest percentage of ewes in estrus was observed between 33 and 57 hours for both study groups (Figure 2). Subsequently, this percentage decreased to its lowest level by 96 hours, with 16.7% for ewes in the SD group and 20.8% for ewes in the LD group.

Ovarian Follicular Development

There were no differences (P>0.05) between the synchronization groups in the number of ovarian follicles (Table 2) at the time of CIDR insertion and removal. Regarding the diameter of the largest follicle, there were no differences (P>0.05) between the groups

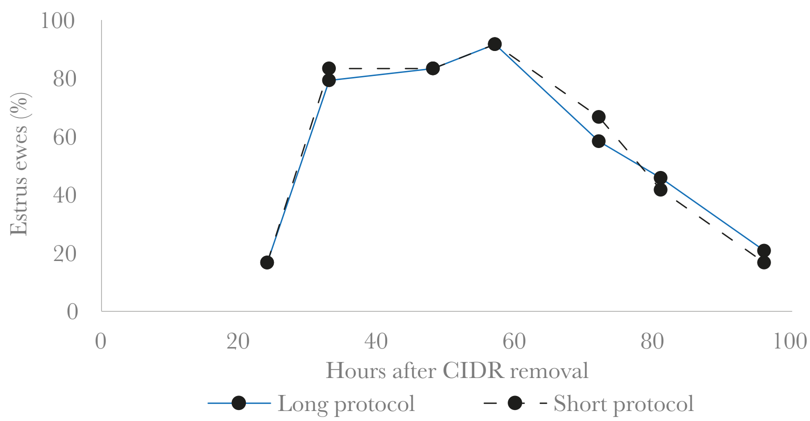

Figure 2. Percentage of Estrus in Ewes with Short (CIDR for 7 days) or Long Duration (CIDR for 12 days) Estrus Synchronization Protocol.

Table 2. Number and Size of Ovarian Follicles at CIDR Insertion and Removal in Hair Sheep with Short (7 days) or Long Duration (12 days) Estrus Synchronization Protocol.

Variable	Short Protocol[1]	Long Protocol[2]
Number of ovarian follicles, LO		
CIDR insertion	2.21±0.21	2.62±0.12
CIDR removal	2.50±0.17	2.37±0.13
Diameter (mm) of largest follicle, LO		
CIDR insertion	5.25±0.36	4.46±0.17
CIDR removal	4.25±0.19[a]	5.42±0.32[b]
Number of ovarian follicles, RO		
CIDER insertion	2.83±0.27	2.67±0.19
CIDR removal	2.67±0.11	2.50±0.15
Diameter (mm) of largest follicle, RO		
CIDR insertion	5.25±0.37	4.50±0.25
CIDR removal	4.29±0.15[a]	5.42±0.42[b]
Total follicles		
CIDR insertion	5.04±0.36	5.29±0.27
CIDR removal	5.17±0.18	4.87±0.21

[1] Insertion of CIDR on day 1, removal on day 7 ± eCG and Cloprostenol; [2] Insertion of CIDR on day 1, removal on day 12 ± eCG. LO=Left ovary; RO=Right ovary. [a,b] Means with different superscripts in the same row are different (P<0.05). Results are expressed as mean ± standard error.

of ewes before CIDR insertion; however, at the end of the treatment, the ewes in the LD group had a larger average diameter than those in the SD group ($P<0.05$).

DISCUSSION

The use of the short-duration estrus synchronization protocol resulted in a reproductive response in ewes similar to that obtained in the group with the long-duration protocol. For many years, protocols have been used in sheep as a tool to help improve reproductive processes. A previous study (Espinosa-Martínez *et al.*, 2020) described the use of short-duration synchronization protocols in hair sheep using progesterone and eCG with good results in estrus presentation (92%). The results of estrus presentation in our study are similar to or better than those reported in the literature (ranging from 70% to 100%), describing protocols with the use of CIDR for varying periods in breeds such as Dorper and Santa Inês (Bruno-Galarraga *et al.*, 2021; Santos-Jiménez *et al.*, 2022; Vinicius *et al.*, 2019).

The onset of estrus in hair sheep in this study showed results similar to (36-41 h; Santos-Jiménez *et al.*, 2022) or different from those observed in other studies, with no consensus as the range varies from 22 to 46 h. This variation could be attributed to the breeds used in the studies and the type of progestogen employed (Alavez-Ramírez *et al.*, 2014; Arroyo-Ledezma *et al.*, 2013; Bruno-Galarraga *et al.*, 2021). The difference in the time periods established for estrus detection likely explains, at least partially, the results obtained; studies with the same frequency of estrus detection as conducted in this work describe similar results (35-42 h; Vilariño *et al.*, 2013). Additionally, another possible explanation could be related to variations in the products used in synchronization protocols; for example, it has been reported that the use of eCG after CIDR removal reduces the onset of estrus (Cox *et al.*, 2012).

On the other hand, the duration of estrus was shorter compared to studies of ewes treated with progesterone (55 h, Alavez-Ramírez *et al.*, 2014; 54 h, Arroyo-Ledezma *et al.*, 2013), although it was longer compared to Dorper ewes (24-29.5 h, Santos-Jiménez *et al.*, 2022). Again, this may be associated with differences in the frequency of estrus detection and even effects related to the breed studied.

The estrus response using a 12-day CIDR was higher than described for hair sheep under tropical conditions using intravaginal sponges (93%, Alavez-Ramírez *et al.*, 2014) and higher than for Dorper sheep with a 7-day CIDR (over 54%, Santos-Jiménez *et al.*, 2022). Additionally, the period of highest estrus response (33-57 h after CIDR removal) was similar to that observed by other authors (Santos-Jiménez *et al.*, 2022). Considering these data, the estrus response obtained with short-duration protocols can be considered appropriate for Pelibuey and Blackbelly genotypes under temperate climate conditions, as observed in their tropical regions of origin.

Perhaps more important than the estrus response is the conception rate achieved with synchronization protocols. This variable also showed good results for both protocols, with rates similar to or greater than those described in previous studies using progestogen protocols (69% in unspecified breed, Bruno-Galarraga *et al.*, 2021; 70% in Ghezel ewes, Hasani *et al.*, 2018; 24-33% in Santa Inês breed, Vinicius *et al.*, 2019). Differences that may exist with other studies could be associated with genotype (Cox *et al.*, 2012), the number of parturitions in the ewe (Santos-Jiménez *et al.*, 2022), type of service (Vinicius *et al.*, 2019), and climate or time of year (Alavez-Ramírez *et al.*, 2014; De *et al.*, 2020).

Small ruminants are mostly seasonal breeders, showing an anovulatory period during long days (Arroyo, 2011). In this study, the good results obtained in the various reproductive indicators could also be attributed to the fact that the ewes were still in the breeding season when the treatments began. An evaluation of their reproductive status prior to the start of the study was not conducted, but the timing is similar to the period routinely used in the production unit for breeding. However, other studies have shown that hair sheep, such as the Pelibuey, can have a short anestrus period (Arroyo, 2011; Valencia *et al.*, 2001), which could also result in lower pregnancy rates in synchronized ewes, as naturally occurs in seasonal sheep (Habeeb *et al.*, 2021; Morris *et al.*, 2004). For this reason, future studies should consider evaluating the reproductive status prior to the use of estrus synchronization protocols. Prolificacy was not affected by any of the treatments, with results similar to those observed in some hair breeds (2.1 lambs in Pelibuey breed, Avendaño-Reyes *et al.*, 2007; 1.9 lambs in Pelibuey breed, Macías-Cruz *et al.*, 2012).

In general, studies do not show information on prolificacy due to the use of progestogens (CIDR) for short or long periods in hair sheep. However, in agreement with this study, dairy ewes treated with CIDR for 6 or 12 days also showed no differences between groups in the number of lambs born (Fleish *et al.*, 2013), which further supports the use of a short synchronization protocol in ewes.

The development of follicles in the ovary is an important aspect to consider in synchronization protocols, as differences in this development could help explain some of the responses to these protocols. In this study, when the devices were removed, the number of follicles was like what has been previously shown in ewes when removing vaginal sponges. The results also show a greater number of follicles compared to studies in ewes (breed not specified by the authors), 24 h after the CIDR was removed (approximately two follicles, Bruno-Galarraga *et al.*, 2021), although without indicating the total at the time of removal. The higher number of follicles observed in the ewes in our study may reflect the prolific condition known for hair breeds like the Pelibuey, predominant (79% in the PC and 83% in the PL) in this study (Aguilar-Martínez *et al.*, 2017).

The diameter of the largest follicle on the day the CIDR was removed was smaller than the follicular diameter described by other authors (greater than 7 mm) in Santa Inês ewes (Oliveira *et al.*, 2016), which could be related to the breed of the ewe or the timing of the ultrasound to determine follicle size. In this study, progesterone concentration in the ewes was not determined; however, it is possible that the larger diameter observed in the ewes treated with long-duration protocols is due to low progesterone concentrations that can occur from 6 or 7 days after CIDR insertion (Arroyo-Ledezma *et al.*, 2013). These low concentrations would allow a higher frequency of luteinizing hormone secretion, which would promote follicular development (Arroyo, 2011). Some studies also describe something similar, ewes treated with intravaginal sponges impregnated with medroxyprogesterone for 6 days had a smaller follicular diameter than those treated for 9 or 12 days (Texeira *et al.*, 2016). In fact, it has been speculated that the decrease in progesterone concentration can affect follicular turnover and stimulate the presence of persistent follicles (Vilariño *et al*., 2013). Under the conditions of this study, the larger follicular diameter in ewes with the long-duration protocol did not represent an advantage in the most important reproductive indicators, such as estrus rate and conception rate. Other studies in ewes have described the strong involvement of plasma progesterone concentrations on some indicators of follicular development, such as the shortening of the growth phase of ovulatory follicles and the maximum follicular diameter, but with no consequence on the ovulation rate (Oliveira *et al*., 2016). In this way, although there are some effects of progesterone concentrations on indicators of follicular development, it does not seem to affect the reproductive response of ewes subjected to the estrus synchronization protocols used in this study, which should be confirmed in future studies.

CONCLUSIONS

In conclusion, short-duration estrus synchronization protocols based on progesterone showed good results in both estrus presentation and pregnancy rate. The different indicators obtained support their use in hair sheep outside tropical regions, under temperate conditions. Additionally, there could be other potential benefits with the use of short-duration protocols, such as reducing production costs, progressing towards minimizing the prolonged use of exogenous hormones for reproductive management, and the possibility of using a CIDR for at least a second application. This is because the devices might maintain a higher concentration of progesterone after removal and using them more than once divides the cost.

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Colegio de Postgraduados

Advances in the Characterization of Creole Cattle from Nayarit, Chihuahua, and Baja California Sur

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ABSTRACT

Objective: This manuscript aims to contribute to disseminating the research conducted on creole cattle from Nayarit, Chihuahua, and Baja California, as these regions have been the focus of numerous studies. These studies include identifying the areas of opportunity that need to be addressed for the conservation of this zoogenetic resource present in Mexico.
Description: Cattle farming in Mexico began with the arrival of the first bovines from the Iberian Peninsula to New Spain over 500 years ago. These cattle established itself in various regions of the country, developing specific characteristics that have allowed it to adapt primarily to harsh environmental conditions. As a result, these bovines are considered a productive alternative in various agroecological environments to address the challenges of climate change and mitigate the negative effects on the ecosystem.
Limitations: To date, research on the morphological, productive, and genetic characterization, as well as the production environment of Creole cattle in Mexico, remains limited. Therefore, it is necessary to develop studies in this area to contribute to the conservation of this resource and, above all, to identify mechanisms for its rational use that justify its existence in a modern economic and productive environment.
Conclusions. The documentary review conducted to present the current state of research on creole cattle herds in Nayarit, Chihuahua, and Baja California Sur revealed that the studies have primarily focused on productive aspects in various environments. Therefore, it is necessary to encourage research directed towards other productive areas of cattle, such as reproduction, animal health, and adaptation, in order to develop strategies that contribute to the conservation of this zoogenetic resource.

Keywords: creole cattle, zoogenetic resources, Coreño, Raramuri, Chinampo.

INTRODUCTION

It is estimated that the global population will grow by approximately 2.1 billion people between 2015 and 2050, which will result in a higher demand for animal-derived food [1]. According to the Second Report on the State of the World's Animal Genetic Resources

for Food and Agriculture, the projected production of ruminant meat is between 60 to 75 million tons between 2030 and 2050 for developing countries, which, due to population growth, may be insufficient [1]. Livestock farming impacts highly relevant sectors such as: food security, the economy, highly marginalized communities, and the cultural development of peoples [1].

Animals, particularly Creole breeds, could adapt to numerous adverse environments, including challenging environmental conditions and areas with scarce food resources [1]. Since the arrival of cattle in Mexico in 1521, they have faced environmental conditions such as the humid tropics, arid environments, extreme temperatures, and new diseases [2]. The first cattle populations from the Iberian Peninsula arrived via the port of Veracruz, spreading across the Mexican plains to New Galicia (now the Sierra Madre Occidental region shared by the states of Durango, Jalisco, Nayarit, and Zacatecas) and New Vizcaya (now Chihuahua) [2,3].

After more than 500 years, natural selection has played a crucial role in enabling these animals to develop traits of hardiness and adaptability [3]. Regarding the conservation of these zoogenetic resources, over the past decade, FAO and SAGARPA have initiated efforts to bring together stakeholders involved in livestock production and the identification of genetic diversity. This led to the development of a report that describes the main breeds present in our country [4], including creole breeds. In the country, there are creole cattle herds in different regions, notably in Chihuahua, Nayarit, and Baja California, which have been studied primarily to determine their productive characteristics.

For example, the Coreño Creole breed is suggested as potentially useful as a maternal breed in beef production when crossed with other breeds [5]. On the other hand, the Creole cattle of Chihuahua represent a genetic alternative for sustainable livestock farming, addressing the challenges of climate change and mitigating the degradation of grassland ecosystems, particularly in the arid and semi-arid regions of northern Mexico and the southwestern United States [6]. As mentioned, despite the academic and research work conducted to date, it is important from a zoogenetic resource conservation perspective to promote efforts to characterize the production systems of these herds. This is essential not only for their conservation but also for their utilization within the production chain. Therefore, the objective of this review is to describe the current state of research advances in the different Creole cattle herds in the states of Nayarit, Chihuahua, and Baja California Sur, as these regions have the most documented information. The aim is to identify areas of opportunity that will enable the development of strategies to contribute to their conservation.

Animal Genetic Resources in Mexico

At both the national and international levels, the demand for animal protein is increasing, necessitating the production of many breeds that are sources of genetic diversity and capable of adapting to the planet's changing conditions [1,7].

In 2012, FAO and SAGARPA presented the Methodological Document for Calculating the Livestock Diversity Subindex. From this report, 54 cattle breeds were identified, including 10 Creole breeds: Creole de Rodeo or Rarámuri, Creole del Golfo, Ganado de Lidia, Creole Chinampo, Creole Coreño or del Nayar, Frijolillo, Cuernos Largos, Creole Lechero Tropical, Romosinuano, Mixteco, and Nunkiní [3,4,8]. In this regard, the National Institute for Forest, Agricultural, and Livestock Research (INIFAP) has three Creole cattle populations: Creole Coreño or del Nayar, Creole de Rodeo or Rarámuri, and Creole Chinampo [9]. In 2007, FAO published "The State of the World's Animal Genetic Resources for Food and Agriculture," which warns that zoogenetic resources are being lost at an accelerated rate. As a result, the publication of the Global Action Plan for Animal Genetic Resources for Food and Agriculture and the Interlaken Declaration was carried out. The Interlaken document establishes as a strategic priority the creation or enhancement of *ex situ* conservation programs and justifies this as follows: "*ex situ conservation measures provide a safeguard against losses of zoogenetic resources in the field, whether due to erosion or as a result of emergencies.*

Ex situ measures complement in situ measures, with which they should be linked where appropriate. ex situ collections can also play an active role in strategic genetic improvement programs" [10]. Depending on their ability to produce offspring with favorable characteristics, various sources of animal germplasm (semen, embryos, oocytes, somatic cells) can be cryopreserved and potentially utilized under different schemes depending on the purpose of regeneration [11].

In this regard, the National Center for Genetic Resources (CNRG) of INIFAP was established as a strategy to contribute to ensuring food security in Mexico through the *ex situ* conservation of cryopreserved animal germplasm, which includes semen, embryos, oocytes, and somatic cells [10]. In the area of zoogenetic resources, semen and embryos of Creole cattle breeds Coreño and Chinampo are preserved. Currently, INIFAP is making efforts to conserve germplasm from different Creole cattle breeds to serve as a backup for the populations in the field.

Creole Cattle Chinampo

The Creole Chinampo cattle of Baja California Sur are cattle whose ancestors were introduced to the Baja California Peninsula in 1697, where the various breeds were exposed to the desert and dry environment of the region for many years [7]. It is a small animal, highly resistant to extreme aridity, where it is capable of surviving and reproducing. It is practically the only breed that survives the harsh environmental conditions of the Baja Californian desert, demonstrating the hardiness characteristics of locally adapted breeds; these cattle are also resistant to both internal and external parasites as well as various diseases. Additionally, it is a docile and long-lived animal [9]. These cattle are primarily used for meat, milk production, and work. Chinampo cattle are employed in extensive grazing systems in marginalized areas that are unsuitable for breeding specialized breeds [12]. Espinoza *et al.* (2011) characterized the coat coloration in Creole Chinampo cattle, finding that the predominant coloration was a combination of white and red, followed by solid red, and then black, white with black, white with tawny, brindle or red striped with black, and other color combinations in lower proportions [13]. Regarding coat colorations, there are other designations such as: Overos, Blacks, Bays, Tawny, Mecos (spotted), Yaguané, BON and BOM, Creams, Browns, Rosillos, and Barrosos [3].

In another study, body weight was also characterized, observing values in females ranging from 115±12 kg for one-year-old animals to 255±9 kg for animals over six years old. On the other hand, the body weight of males varied from 130±8 kg in one-year-old animals to 345±15 kg in bulls of four years or older. In general, the average weight of females is 255 kg, and 345 kg for males [14].

Regarding hair length, three categories were determined: 1) short hair estimated at 0.5 cm or less; 2) medium hair from more than 0.5 cm to one centimeter; and 3) long hair of 1.1 cm or more [3].

Concerning the tail tassel, it was classified as follows: 1) short tassel (but not absent, which is a trait, rat tail, associated with thermoregulation in brindle cattle); 2) medium tassel; and 3) heavy tassel, typical of *Bos taurus* of European origin and present in current Spanish breeds; indicating poor adaptation to the tropics [3].

On the other hand, the estrous behavior of the cows was studied, and it was concluded that, in the presence of the male, the cows concentrated their estrous behavior into a shorter period and increased the proportion of estruses with a duration of <8 hours. The presence of the male influenced the onset time of estrus, which occurred in the morning [13].

Regarding the suckling habits of Creole Chinampo calves, it was observed that they follow a pattern similar to that reported in other beef breeds (with higher frequency of suckling during the early morning and afternoon hours). Additionally, the frequency and duration of suckling decrease by 180 days. It was also found that females suckled more frequently than males [15]. In another study, the response to physiological variables (rectal temperature and respiratory rate) related to heat tolerance was compared between Chinampo and Jersey or Holstein cows, concluding that Chinampo cows were more heat-tolerant than Jersey or Holstein [16,17].

A broader study on the genetic characterization of 26 Creole cattle breeds from 10 Latin American countries included five Creole breeds from Mexico [16]. These authors used 19 microsatellites to estimate genetic diversity as a basis for implementing conservation and sustainable management programs. They reported that, in the case of Mexican cattle, Creole breeds from Puebla, Chihuahua, Baja California, and Nayarit formed a cluster with the Creole Cuernos Largos from South Texas, indicating a close genetic relationship between populations from geographically adjacent regions [18].

Creole Coreño Cattle

The Creole Coreño cattle are found in the Sierra Madre Occidental region shared by the states of Durango, Jalisco, Nayarit, and Zacatecas, and are generally managed by indigenous Coras, Huicholes, and Tepehuanos [19]. This cattle is primarily used for the production of cheese and meat for self-consumption, in addition to being used as support animals in agricultural work due to their docile temperament under frequent handling conditions [19].

In 1982, a Coreño Creole cattle herd was established with the support of INIFAP and the Board of Trustees for Livestock Research (Patronato para la Investigación Pecuaria - PAIPEME) of organized cattle producers [3]. This herd originated from the acquisition of 50 cows and 10 bulls from the Sierra Madre Occidental region, which were settled at the El

Verdineño experimental site of INIFAP, located in the municipality of Santiago Ixcuintla, Nayarit, at 21° 42' north latitude and 105° 07' west longitude, with a tropical climate, an altitude of 60 meters above sea level, and an average annual temperature and precipitation of 24 °C [20]. With the establishment of this herd, studies began on the productive, reproductive, and genomic characterization of Creole Coreño cattle, seeking to assess the potential of this breed. For more detailed information on these investigations, refer to Martínez *et al.* (2021), who conducted a comprehensive review on the characterization of Creole Coreño cattle. Therefore, only notable points are addressed here [19,21].

In reproductive characterization, comparative studies between Guzerat heifers and Creole Coreño heifers in tropical conditions have shown that Creole Coreño heifers had their first calving earlier (1,392±38 days) compared to Guzerat heifers (1,655±31 days) [22]. This indicates greater reproductive efficiency in Creole Coreño heifers compared to Guzerat, as heifers that calve at a younger age produce more calves during their productive lifespan [23]. Furthermore, it was found that Creole Coreño cows had higher rates of conception, calving, and weaning (60%, 59%, and 52%, respectively), compared to Guzerat cows (46%, 42%, and 38%, respectively) [24]. On the other hand, superovulation studies in Creole Coreño heifers and cows demonstrated that it is feasible to use reduced doses of FSH (140 to 200 mg) without affecting the response to superovulation or embryo production [25]. In this same study, embryo production ranged from (1.1±0.7 to 2.9±0.7), indicating that Creole cattle, on average, have a lower production of transferable embryos compared to other breeds such as Nelore (10.3±10.3) and Red Angus (5.9±1.1) [25,26,27]. This may be due to endocrine failures caused by ovarian overstimulation resulting from superovulation protocols when applied to small-sized females; additionally, older females show decreased embryo production [25].

On the other hand, in terms of productive characterization, the production and composition of milk have been evaluated, revealing lower total and daily milk production in Creole Coreño cows (805 kg and 3.90 kg, respectively) compared to Guzerat cows (949 kg and 4.5 kg, respectively) [18]. This could explain the lower weaning weight of Creole Coreño calves (153.50 kg) compared to Guzerat calves (177.42 kg) [5].

Research conducted at INIFAP suggests that this cattle breed may play a significant role as a maternal breed in crossbreeding with bulls of other breeds within the context of commercial beef production. This is since females of this breed exhibit better fertility and maternal ability, which can translate into an increase in weaned calf weight per cow [5,28]. Additionally, it has been observed that carcasses from steers derived from Creole Coreño cows, Creole Coreño × Guzerat crosses, and Guzerat × Creole Coreño crosses inseminated with Angus bull semen are of good quality and performance compared to steers derived from Guzerat cows inseminated with Angus bull semen [5].

In the context of physical characterization, there is limited information regarding morphological measurements and phenotypic characteristics of Creole Coreño cattle. Available data include birth weight (29 kg) and weight at 3.5 years (500 to 550 kg) [3]. Regarding coat color, Alba (2011) describes 28 animals observed at El Verdineño, and photos of animals at Mesa de Nayar, finding 10 bayos (3 hoscos, 2 zainos, one with a white breastbone, 2 with a star, and 2 with white and speckled arlequin patterns of the base color), 2 reds (both with a star and white belly), 1 brown (with a lighter dorsal stripe), 1 barcino (or meco), 7 overos (2 red, 3 black, and 1 bay), 1 BON (partially dominant white with black ears), 1 with a black lateral color, 2 blacks (one zaino and another with white spots on the belly), and 2 barrosos (typical black dilution) [3].

Although limited information has been generated regarding these cattle, the Association of Mexican Creole Cattle Breeders (Asociación de Criadores de Ganado Criollo Mexicano, A.C.) states that the Coreño Creole cattle meet the phenotypic description of Creole cattle in Mexico [29].

Additionally, molecular characterization studies of Coreño Creole cattle have been conducted. In a study on genetic diversity using SNP markers in Coreño Creole cattle from three locations in Nayarit —El Nayar, La Yesca, and Santiago Ixcuintla— it was found that they maintain high levels of genetic diversity and that the El Nayar population is distinct from the other two according to the estimated molecular coancestry, allowing them to be considered as two separate populations [30]. Another genetic diversity study using microsatellites showed that there has been considerable genetic differentiation among Creole cattle populations from Nayarit, Chihuahua, Durango, and Guerrero. Additionally, it was found that there is no evidence of *Bos indicus* influence in the Creole cattle from Nayarit [31], suggesting that it could be considered a well-defined Creole breed.

Unfortunately, a decrease in these cattle populations is estimated, which may be attributed to the introduction of *Bos indicus* breeds into the region [32,3]; currently, the status of Coreño populations is unknown.

The livestock industry in Chihuahua thrived with cattle descended from breeds that arrived from Spain, which had the ability to adapt to different environmental conditions and provided multiple services (meat, milk, draft power, hides, and tallow) to the growing population [30,31]. However, due to the increased demand for beef in the United States and the demarcation of lands in the region, the introduction of specialized meat breeds began in the late 19th century. This led to the decline of the Creole cattle population in Chihuahua, until it practically disappeared from regions where the main ecosystem is grassland [33,34].

Creole Cattle of Chihuahua

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At the end of the 90s, the Autonomous University of Chihuahua (UACH) [34] and the Experimental Ranch La Jornada (ERL) of the United States Department of Agriculture (USDA) in collaboration with New Mexico State University, in 2005 [33], decided to introduce a herd of Creole cattle, selected directly from the Sierra Tarahumara, not only with the aim of preservation but also for research on grazing behavior, economics, as well as productive, reproductive, and meat quality characteristics [33,34]. All this was with the purpose of demonstrating the characteristics of the Chihuahua Creole cattle that would allow its introduction into the beef production system, either as a pure breed or in various breeds.

Quintana-Gallegos and collaborators (2016) mention that the main advantages documented for the Chihuahua Creole cattle compared to European cattle specialized in meat production are: their small size, which implies lower nutritional requirements to meet their physiological needs [38], and their different grazing habits or patterns, which give them the ability to meet those nutritional requirements with less negative impact on the ecosystem [36] (Roacho-Estrada, 2023).

Quintana-Gallegos *et al.* (2016) found that F1 Angus × Creole cows in the early lactation stage had a lower weight compared to Hereford × Angus cows, as analyzed by the regression equation ($0.18x+328$ and $-0.33x+383$, for the former and latter, respectively) of body weight (kg) in the 70 days postpartum (day zero) [35]. Additionally, they observed that the concentration of β-hydroxybutyrate (an indicator of lipid catabolism) did not change over the days during the last third of gestation and early lactation (0.0001±0.0002 mmol/L/d), whereas in the case of Hereford × Angus cows, an increase was observed ($P<0.01$; 0.0011±0.0002 mmol/L/d) during the same period. These results indicate that the lower weight and advantages in grazing habits of Angus × Creole cows make mobilization more efficient and/or reduce excessive loss of energy reserves. During periods of lower forage availability and quality, Creole cows have more extensive exploration patterns compared to the Angus breed (6.7±0.56 and 5.4±0.56 km/d for Creole and Angus, respectively).

On the other hand, during periods of higher forage availability and quality, there are no differences between the two breeds. Additionally, the slope traversed, and the elevation reached by Creole cows were greater compared to Angus cows, regardless of the season [36]. The study found that European cattle spent more time grazing in open medium grassland compared to Creole cattle (10.7 *vs.* 9.9 h d^{-1}). Both breeds showed increased grazing time during periods of food scarcity. The grazing patterns and diet diversification generate differences in the ruminal microbiota of the animals; this was corroborated by Maynez-Perez *et al.* (2021) through a comparative metagenomic analysis of the 16s ribosomal gene between Creole and European cattle [37].

In the United States of America, a specialized market has been created for the meat of these animals [38]. However, such a market does not exist in Mexico. In this regard, the UACH has proposed a three-breed crossbreeding strategy, where F1 Angus × Creole cows are used as dams on the range, as it has been proven that the ecological behavior advantages of pure Creole are retained in these F1 cows.

Finally, these cows would be crossed with a terminal meat breed, and all offspring would be destined for supply [39]. This study shows how Piedmontese × Angus × Creole animals were highly competitive in terms of slaughter weight, carcass, and meat characteristics. An interesting aspect is that the meat from animals with a Creole component had less cutting effort (0.92±0.18 kg force compared to Hereford × Angus and 0.78±0.2 kg force compared to Piedmontese × Hereford × Angus). This trait had already been observed in meat from pure Creole animals, meaning that Chihuahua Creole animals produce tender meat (3.75±0.08 kg force) [40].

Regarding reproductive characteristics, a study conducted in Ciudad Juárez, Chihuahua, found that the majority (73.3%) of Creole females exhibited two waves of follicular development per cycle, demonstrating a good ovarian response. Although the size of the corpus luteum and follicles were smaller (13.0±1.0), the hormonal levels were ideal for pregnancy (6.5±0.1 ng/mg) [41]. The semen from Creole males in a confined system showed individual motility above 80% and blood testosterone levels above 3.8 ng/mL, with these values remaining constant throughout the year. In contrast, European breeds exhibited a decrease in individual motility and testosterone levels during the hot season, reaching values of 58% and 2.8 ng/mL, respectively [42].

In a grazing system with low inputs, the levels of mRNA PLCZ1 (related to fertility percentages) in the sperm of Creole bulls tended to be higher compared to Angus bulls both in winter and summer. These levels were 5.3 times higher ($p<0.05$) in winter than in summer. This indicates that environmental conditions, availability, and quality of forage can affect biochemical and molecular processes related to fertility traits and reproductive efficiency. However, further studies are needed in this regard.

Colloquially, the resistance of Creole animals to diseases has been mentioned, although studies on Creole cattle in Chihuahua are virtually nonexistent. Despite this, high diversity in the BoLA-DRB3.2 gene has been observed, and some allelic variants that had not been previously reported in other populations have even been identified [43,44]. This suggests that this cattle breed may have a higher potential to respond immunologically to a broad range of antigens.

On the other hand, as a result of combined efforts, INIFAP together with the Mexican Association of Creole Cattle Breeders (ASOCRILLO) established the Creole cattle herd at the La Campana Experimental Ranch.

In this regard, an evaluation of heifers during the growth stage showed an average daily weight gain of 680 g per day, 800 g during the development stage, and 680 g per day during finishing. As for feed conversion efficiency, the rates were 8.8, 9.4, and 8.9, respectively. In this study, the average slaughter weight was 271 kg, with a hot carcass yield of 52.70% and a cold carcass yield of 52.30% [45]. Regarding the physicochemical characteristics of the meat, *e.g.*, the luminosity was 47.7, the redness tendency was 15.5, the yellowness tendency was 17.5, water-holding capacity was 64.7%, and pH was 5.7. These values do not allow for discrimination between meat from commercial cattle and meat from Creole cattle [46].

Recently, at the La Campana Experimental Ranch, research activities with this herd have resumed. One of the main areas of interest is studying the composition of the rumen microbiota and methane emissions, in addition to analyzing the expression of genes related to heat stress, as has been done with other herds at INIFAP.

The objective of this manuscript was to present the research developed on Creole cattle, particularly in the states of Baja California, Nayarit, and Chihuahua, with the aim of identifying areas of opportunity that remain pending, such as associations between grazing patterns, rumen microbiota findings compared to other breeds, disease resistance, etc. The latter, by utilizing current molecular tools and omics sciences, studies in metagenomics, transcriptomics, etc., could be conducted to make associations with productive parameters. This would contribute to a better characterization of this resource in Creole cattle in Mexico and provide suggestions for its conservation.

CONCLUSIONS

Locally adapted zoogenetic resources are generally neglected in terms of conservation efforts, making them vulnerable and at risk of disappearing. This review has presented the documented information on Mexican Creole cattle, demonstrating that there are many opportunities, particularly from a productive aspect, as some herds have been more extensively characterized. Therefore, it is essential to support the formation and/or strengthening of specialized study groups for them *in situ* and *ex situ* monitoring and conservation. Prioritizing the genetic value of these resources through cryopreservation of animal germplasm and storage in germplasm banks is crucial. In this context, research generated by INIFAP, and other educational institutions is vital for the conservation and utilization of this zoogenetic resource in Mexico, considering factors such as population size, genetic diversity, productive behavior, and the characterization of the production system.

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Colegio de Postgraduados

Time to flowering of ornamental orchids

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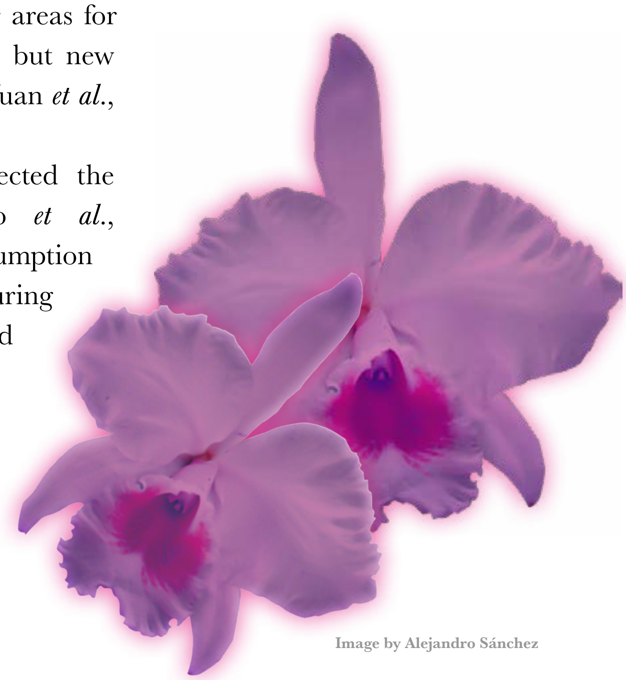
ABSTRACT
Objective: To analyze features of time to flowering of orchids and some strategies for its control in cultivation for ornamental purposes.
Design/methodology/approach: Information from various authors and our own data is shown, in relation to the management of flowering time of ornamental orchids, based on treatments of environmental effects, plant growth regulators (PGRs) and nutrition.
Findings/conclusions: It is possible to reduce the flowering time of orchids by environmental modifications, mainly temperature and photoperiod. This can also be achieved by nutritional management, mainly macro-nutritional, and through organic nutrition and PGRs. However, much more information is needed, especially for native orchid species with ornamental potential.

Keywords: Management of environmental factors, nutrition, orchid cultivation, ornamental orchids, plant growth regulators.

INTRODUCTION

Orchids as potted and cut flowers represent a large segment of the floriculture industry, due to their spectacular blooms. The value of world flower production is estimated at US$ 55 billion [Rabobank Food & Agribusiness Research and Advisory y Royal FloraHolland (Consulta: enero, 2022)]. Thus, the cultivation of orchids on a large scale as cut flowers and in pots continues to be a trend, and they head the highest prices in the world market (Hew y Yong, 2004; Rabobank, 2016; 2022). Southeast Asian countries are the major players in the global orchid trade, among which Thailand is the largest producer, with USD $60 million in 2014 (Thammasiri, 2016). The European Union (EU) and the United States are the most important countries or areas for the commercialization of orchid products, but new orchid markets are increasing every year (Yuan *et al.*, 2021).

While the COVID 19 pandemic affected the floriculture production sector (Anacleto *et al.*, 2020), there was an increase in the consumption preference of horticultural products, as during the pandemic quarantine, people encouraged the cultivation of ornamental plants at home (Pérez-Urrestarazu *et al.*, 2021). Likewise, the orchid trade has increased through digitalization or electronic commerce (E-commerce), coexisting with traditional flower shops



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and supermarkets (Yuan *et al.*, 2021), and consumer preference for continuing to purchase orchids has increased in some countries, such as Indonesia (Nadzifah *et al.*, 2022). *Phalaenopsis* is the world's most commercialized orchid with tremendous technological advances in breeding and micropropagation in several countries, such as the Netherlands, Taiwan, and Thailand, among others, for its ease of controlled flower induction for scheduled and year-round production (Yuan *et al.*, 2021). In the future, greater production and demand for orchid products are expected to arise from non-conventional technologies, such as efficient biotechnological breeding, (Yuan *et al.*, 2021), or from the introduction of wild species as new ornamental products, among which many species have been successful (Darras, 2020), and of which orchids should represent a fundamental group.

From a commercial point of view, one of the problems with orchids is that they have long juvenile periods before reaching the reproductive stage. Some important commercial orchids can take 2 to 3 years, such as *Phalaenopsis* (Wang *et al.*, 2017), but others take extreme periods, such as Aranda, which can require up to 13 years (Hew and Yong, 2004; Huang *et al.*, 2021). Likewise, some species have irregular or long flowering cycles throughout the year, so that alterations in the weather, for example, make it difficult to make materials available at times of high market demand (Hew and Young, 2004.). Thus, the long wait for flowering is a disadvantage for their success in the market, so the regulation of flowering time is the key to studies on the floral development of orchids (Ahmad *et al.*, 2022).

Scientists are therefore increasingly interested in better understanding the mechanisms and factors that influence the vegetative to reproductive transition in orchids to reduce their time to flowering, shorten production cycles, and thus reduce their time in the greenhouse and their use of agrochemicals and energy. Based on genetic, physiological and technological studies, various techniques are available to control the time to flowering, hastening or retarding the growth of the plant and its rate of development to adapt flowering to market demand (Proietti *et al.*, 2022). In this paper we reviewed current information on flowering time in orchids, as well as some approaches to its control for the cultivation of ornamental orchids. In addition, data derived from our research are incorporated.

Flowering of orchids

Orchid flower structure is uniquely diversified among flowering plants, and most orchid species have defined seasons favourable for flower induction and development, but there are many unknown features beyond our understanding of the orchid flower transition phenomenon (Wang *et al.*, 2017). A better understanding through different biotechnological, genetic and molecular techniques facilitates the modification of desirable flowering characteristics to promote ornamental breeding programs (Li *et al.*, 2021; Liyama *et al.*, 2024). There is some information in the literature on how to manipulate flower induction in orchid species, nevertheless, the lack of scientific information on the control of flowering of most orchids limits greenhouse growers and hobbyists to flower their plants outside their natural flowering period (López y Runkle, 2005).

Time to flowering of orchids

Each plant species, herbaceous or woody, has its characteristic juvenile cycles, during which, even when plants are provided with favourable conditions for flowering or treated with horticultural methods, it is difficult to induce the beginning of flowering (Tsai y Chang, 2022). Orchids present the greatest problems in this regard because of their long juvenile cycles that can range from 3 to 13 years before reaching the reproductive stage (Hew and Yong, 2004). For example, the duration of the juvenile phase in some Vanda hybrids can range from 3 to 8 years; similarly, some *Aranda* hybrids can range from 4 to 13 years. For *Laeliocattleya* Cheah Chuan Keat 6.7 years of juvenile period duration is reported (Hew y Yong, 2004).

Therefore, manipulation of flowering time and frequency of flowering is essential to increase the ornamental value of orchids (Ahmad *et al.*, 2021). Time to flowering is one of the most important horticultural characteristics of orchid cultivars, and a stable flowering time is the main objective of breeding programmes to induce horticultural novelties in commercial crops (Ahmad *et al.*, 2022).

According to Hew and Yong (2004) the flowering routes of orchids are divided into 7 groups : i) Year-round free flowering (*Arundina graminifolia*) (Ahmad *et al.*, 2021); *Prosthechea cochleata**; ii) long flowering season with short to medium non-flowering intervals (*Laelia anceps**); iii) seasonal, flowering mainly during the dry season (*Vanilla planifolia**; *Oncidium sphacelatum**); iv) seasonal, flowering mainly during the rainy season (*Stanhopea* sp.); v) regular in flowering; vi) sporadic at flowering; vii) spontaneous flowering (*personal observations of some species). Induction and seasonality of flowering are significant factors that determine the price and marketability of a popular orchid cut flower. Therefore, a precise recording of the flowering months is essential in a commercial crop. (Hew and Yong, 2004). *Phalaenopsis*, the most traded genus worldwide, usually reach maturity after three to five leaves have developed, which can take a few years (Wang *et al.*, 2019).

In a study of the time-phenological phase of *L. anceps* subsp. *anceps*, Tejeda-Sartorius *et al.* (2017) reported that the period to reach anthesis of the first raceme flower, measured from the visible inflorescence (VINF), 10 cm in length above the pseudobulb apex, is around 130 days on average. Subsequent observations show that to this period must be added another 20 days, on average, which is the time it takes for the flower stalk to reach the indicated length of 10 cm from the time it is just visible. In addition, to this period must be included the time from flower induction in the apical meristem of the stem (pseudobulb), with the signs of flower differentiation, which has not been determined. Thus, considering the periods of visible inflorescence onset, a 150-day cycle mean is established for *L. anceps* subsp. *anceps* (Figure 1), which may vary depending on climatic conditions or management. Based on this characterisation, *Laelia anceps* subsp. *anceps* is in the classification "long flowering season with short to medium non-flowering intervals".

Flowering time management

Time to flowering is controlled by endogenous genetic cues, influenced by plant age, hormones and circadian rhythm, as well as by environmental conditions such as day length, temperature and different types of stresses (drought, salinity, pathogens, water

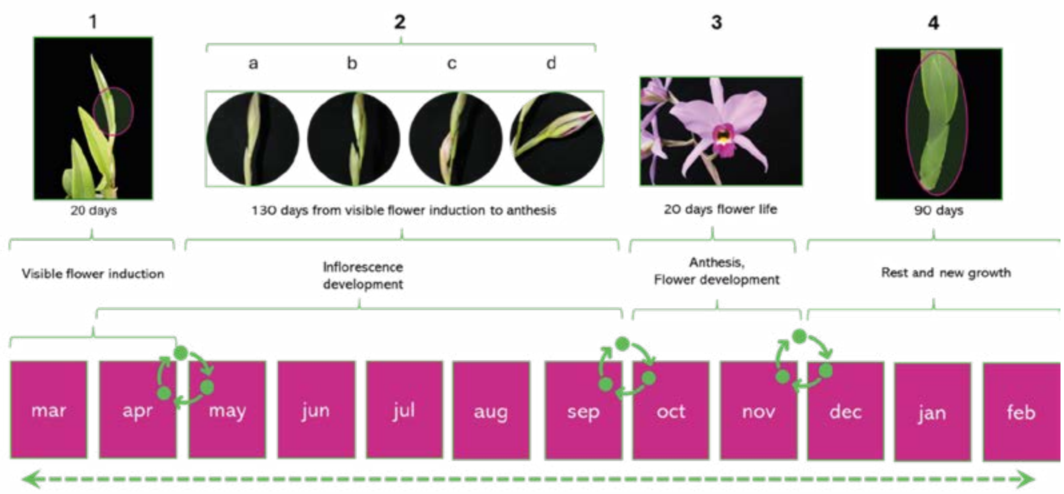


Figure 1. Flowering cycle of *Laelia anceps* in cultivation: 1: 20 days to visible flower induction when the inflorescence has reached 10 cm in length above the pseudobulb apex (pink circle). 2 a-d: 130 days includes elongation of the flower stalk and advancement of flower bud filling until the flower bud opens, and the labellum becomes visible. 3: 20 days flower life is the average derived from several flowers in different flowering cycles, without applications of plant growth regulators or any other bloom inducing substance or treatment. 4: 90 days of vegetative rest (mainly December and January) and in February the formation of new vegetative shoots begins (pink circle), which will give rise to the next flowering. Arrows in circles indicate possible temporal alterations in the different stages of the flowering phenology caused, for instance, by alterations in environmental conditions or management. Dotted arrow indicates repetition of the annual vegetative-reproductive growth cycle.

availability, etc.) (Amasino and Michaels, 2010; Cho *et al.*, 2017). In addition, flowering time is influenced by nutrient availability and exogenously applied chemicals (Cho *et al.*, 2017). Flowering of orchids responds in a similar pattern to that of other angiosperms.

Role of environmental factors in the orchid flowering

Light effect. Kim *et al.* (2015) reported that a night interruption (NI) period with high light intensity (120 μmol m^{-2} s^{-1}) reduced the time to visible buds and flowering of different *Doritaenopsis* cultivars more than NI with low light intensity (3 to 7 μmol m^{-2} s^{-1}). And they suggested high light intensity strategies in *Doritaenopsis* cultivation to obtain early flowering with high quality plants, using the IN treatment during their reproductive stage. Lee *et al.* (2019) found that during forced conditions for flowering of *Phalaenopsis* Queen Beer Mantefon, irrespective of the applied photoperiod [(8/16, 16/8 or '8+8' light/dark)] the highest applied light intensity (300 μmol m^{-2} s^{-1}) decreased the time to visible inflorescence by approximately 20 days compared to 75 μmol m^{-2} s^{-1}.

Efecto de temperatura. Lin *et al.* (2011) found that at least four weeks of chilling at 10 °C with light is needed for full flowering initiation time of *Dendrobium* Red Emperor 'Prince; while Den. Sea Mary 'Snow King' and Den. Love Memory 'Fizz' only needed two weeks of chilling at 10 °C, regardless of light. In other work, Han *et al.* (2020) investigated different temperatures and photoperiods in the regulation of flowering time of *Anoectochilus roxburghii* (Orchidaceae) and found that flowering time can be early under long day conditions of 16/8 h (day/night) and 25/20 °C (day/night). But it is delayed under short day conditions, 8/16 h at 20/15 °C.

In some work with *Laelia anceps*, comparisons were made between i) environment 1, with light combining high-pressure discharge (HID) lamps: metal additive (MA)+high-pressure sodium (HPS)+fluorescent lamps (F), with an intensity of 80 μmol m^{-2} s^{-1}; ii) environment 2, with light emitting diode (LEDs), red/blue (90/10%), 64 μmol m^{-2} s^{-1}. Both environments with day/night temperature 25/13 and 25/17 °C, and photoperiods 9/15 and 12/12 h. We found that environment 1 reduced the time to anthesis by 35 to 75 days (38 and 54%) at 25/17 °C, compared to 25/13 °C, regardless of photoperiod or type of light environment (Figure 2). It is observed that a higher night temperature (17 °C) is necessary to decrease the time to anthesis, or it may be the effect of a lower day/night temperature difference of 8 °C compared to the day/night difference of 12 °C of the other temperature tested (25/13 °C) (Figure 2), (unpublished data).

Furthermore, when comparing greenhouse and controlled conditions of light and temperature in flowering of *L. anceps*, it was found that the time to visible flower induction (VFI) decreased by up to 43 days in the controlled conditions (Sánchez-Vidaña *et al.*, 2018). Once VFI was present, flower development was promoted in the greenhouse and greater uniformity of flowering was observed. Thus, temperature and photoperiod in controlled environment can help not only to reduce the time to anthesis, but also to uniform floral development.

Chemical control of orchid flowering time

Plant growth regulators

A further alternative to plant breeding methods is the chemical engineering of flowering time by external application of flowering-inducing chemical compounds (Ionescu *et al.*, 2017). Among these, phytohormones and their synthetic compounds have long been used in the horticultural industry (Lee *et al.*, 2021), and have important application in the regulation of orchid flowering. Of these, cytokinins (CKs) and gibberellins (GAs) are of the most important, with possible effects, depending on the species, on flower induction and flower stalk elongation, as well as on time to flowering (Ahmad *et al.*, 2022; Yin *et al.*, 2022). Blanchard and Runkle (2008) reported shortening the time to visible flower

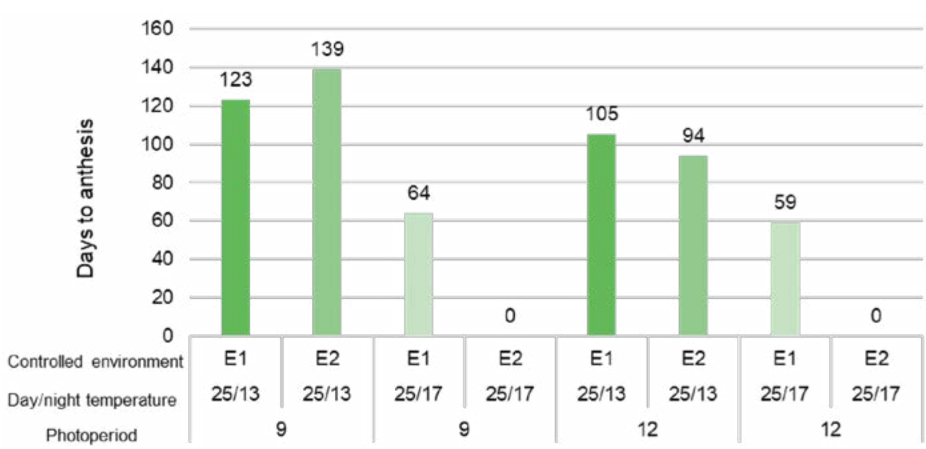


Figure 2. Days to anthesis of *Laelia anceps* under the influence of two controlled environments (E) of light and temperature: E1) fluorescent + metal additive + high pressure sodium; E2) light emitting diode (LED), red/blue.

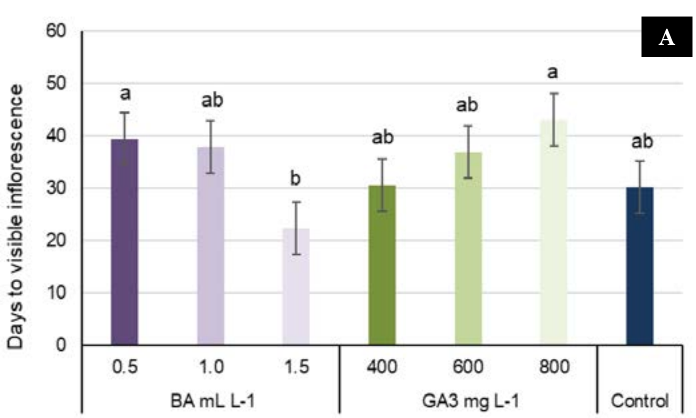
induction from 2 to 6 days in *Phalaenopsis*, using GA_3 and BA. Similarly, Nambiar *et al.* (2012) found that BAP promoted earlier flowering in *Dendrobium* hybrid (Dendrobium Angel White). In a study to evaluate exogenous application of gibberellic acid (GA_3) and 6-benzylaminopurine (BAP) in *Phalaenopsis*, cytokinins were associated with rupture of axillary vegetative meristems and inflorescence, but exogenous GA_3 spraying did not improve inflorescence initiation (Lee *et al.*, 2021). Yin *et al.* (2022) found that exogenous application of gibberellin (GA_3) promoted flowering of *Paphiopedilum callosum* by inducing early bud break. At different doses of BA and GA_3 we have found that the higher the concentration of BA, the fewer days to visible inflorescence. On the contrary, both days to visible inflorescence and days to anthesis increase with higher GA_3 concentration (Figure 3).

Likewise, in a study of three flowering cycles, Tejeda-Sartorius *et al.* (2021) reported that different doses of GA_3 and BA reduced days to visible flower induction and days to anthesis of *L. anceps*. The reduction of the complete flowering cycle was between 3 to 4 months, which is a very considerable time given the long flowering cycle of the species. Positive correlations have been observed between flower spike length and time to anthesis, as well as higher flower spike strength, among other characteristics of flower development (higher number of flower spikes and flowers), compared to the control (Figura 4).

Other regulators are also being tested, such as paclobutrazol (PBZ), a plant growth regulator with gibberellin inhibitor function (Desta and Amare, 2021), which is showing that when PBZ is sprayed on different sized stalks, the smallest ones (between 5 and 15 cm) accelerate their flowering, being very similar to that of stalks larger than 30 cm. This means that flowering is advanced, with reduction in the length of the flowering stalk (unpublished data).

Nutrition management in time to flowering

Inorganic nutrition. The nutritional management of orchids is more complicated than any other agri/horticultural crop, especially in epiphytic orchids, because of the nutrient and water scarcity conditions to which they are often exposed (Biswas *et al.*, 2021). To face



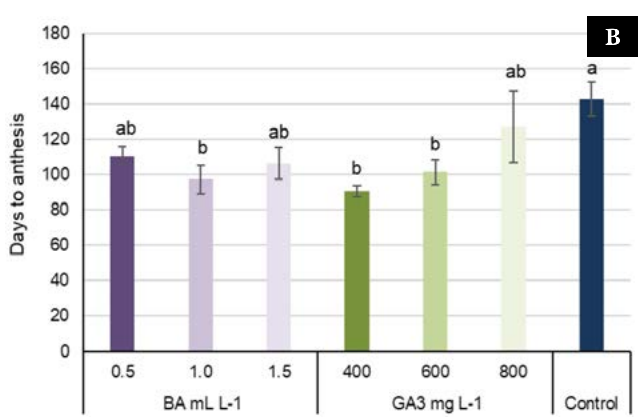


Figure 3. Days to visible inflorescence (A) and anthesis (B) of *Laelia anceps* with different concentrations of benzyladenine (BA) and gibberellic acid (AG_3). Means ± SD with different letters indicate statistical differences between treatments (Tukey, $P \leq 0.05$). Control without plant growth regulators.

Figure 4. Flowering of *Laelia anceps* treated with next doses of plant growth regulators (PGRs) (mg L^{-1}): A) 19 BA; B) 600 AG_3; C) 2.37 BA + 100 AG_3; D) Control plants were sprayed with distilled water and without spraying PGRs. The dose BA + GA_3 (C) showed the greatest reduction in the time to anthesis, *i.e.*, 44 days earlier than the control (D). However, the other treatments also reduced the time (21 days mean) *vs*. the control. Furthermore, both the number of flowering spikes and the number of flowers in A, B and C are noteworthy in relation to the control (D) Source: Data from Tejeda-Sartorius *et al.* (2021).

adverse environmental conditions, orchids have developed highly specialized mechanisms, such as the root velamen, which can absorb water and nutrients quickly and retain them to reduce losses (Zots & Winkler *et al.*, 2013). Research related to mineral fertilisation in orchids is scarce, but orchids are considered to have nutritional requirements like other species (Ichinose *et al.*, 2018; Biswas *et al.*, 2021). Thus, nitrogen nutrition is an essential practice for orchid cultivation (Mantovani *et al.*, 2018) and can help growers to produce orchids more efficiently (Zong-min *et al.*, 2012). But growers generally use mineral nutrition based on other agricultural crops, without considering the specific nutritional needs of each species (Costa *et al.*, 2024). Mineral fertilization, together with substrate mixtures, are very important for vegetative growth to have quality blooms. For *L. anceps*, we obtained early inflorescence emergence in substrates with pine bark (PB) and 200 mg L^{-1} N, or when PB was combined with other substrates. Likewise, there is considerable decrease in the time to inflorescence when lower proportions of PB are present in the mixture (50%) in relation to peat moss (PM) or coconut fibre (CF) (Cuadro 1).

Wang (2007) showed that *Phalaenopsis* Taisuco Kochdian plants treated with 200 mg L^{-1} of N and P, but with low doses of K (50 and 100 mg L^{-1}) in substrate with only pine bark, advanced their flowering, approximately by 15 days, compared to plants grown with moss. Themselves, Zong-min *et al.* (2012) reported that *Paphiopedilum armeniacum* with a high dose of N (420 mg L^{-1}) decreased the time to flowering by five days compared to the lower dose (105 mg L^{-1}), and 8 days when no N was applied. Satari *et al.* (2022) observed no difference in time to first flower opening of *Phalaenopsis*, with application of various NPK formulations (19-6-20, 12-12-36, 20-20-20), where the range of days to anthesis was 278 to 282 days. Ruamrungsri *et al.* (2021) found a positive correlation between K concentration and days to flower opening of Vanda 'Manuvadee'. Tsai and Chang (2022) found no positive linear correlation between C/N ratio and days to inflorescence, or days to first visible bud or first anthesis. For *Laelia anceps* we have found a decrease in time to

Table 1. Interaction of study factors on days to visible flower induction of *Laelia anceps*, by effect of different substrate blends, proportion of pine bark in the substrate and nitrogen levels.

Substrate	**N Fertilisation ($mg\ L^{-1}$)**	**Days to visible flower induction**
PB+P	100	101.3 a
PB+P	200	65.9 b
PB+PM	100	77.8 b
PB+PM	200	77.9 b
PB+CF	100	39.1 c
PB+CF	200	26.9 c
Proportion of CP in substrate (%)		
75	100	142.8 a
75	200	105.3 ab
50	100	25.6 c
50	200	22.8 c
25	100	49.8 bc
25	200	42.7 bc

Means with different letters in the column are statistically different (Tukey, 0.05).
PB: pine bark; PM: peat moss; CF: coconut fibre.

flowering (up to 50 days) or earlier flowering (in June-July) or outside the normal flowering season (October-November) with an NPK fertilisation programme: high N application in vegetative phase, with a change to high P and K application in flowering inductive stages.

Organic nutrition. There are specific organic orchid fertilisers on the market, some of which greatly stimulate rooting and vegetative growth (Rodrigues *et al.*, 2010), but there is little research on the use of organic fertilizers or biofertilizers for orchid growth and flowering (Tejeda-Sartorius *et al.*, 2013). Some authors have tested these products, with better results when combining mineral and organic fertilization (Rodrigues *et al.*, 2010; Tejeda-Sartorius *et al.*, 2018; Biswas *et al.*, 2021) or when applied separately (Tejeda-Sartorius *et al.*, 2013). Significant effects on physiological parameters during the vegetative growth phase of some orchids have been found (Tejeda-Sartorius *et al.*, 2018; Hoshino *et al.*, 2021), and the positive effects could be observed later in the flowering time and reproductive stage of these species.

There are reports of other products considered as biostimulants, explicitly defined as "substances or micro-organisms applied on plants in order to improve nutritional efficiency, abiotic stress, regardless of their nutrient content" (du Jardin, 2015). These include humic and fulvic acids, seaweed extracts, beneficial fungi and bacteria, composts and humus-derived substances, etc. (du Jardin, 2015; Bose y Pal, 2023), of which positive effects on orchid growth and flowering have been reported (Biswas *et al.*, 2021). Some biofertilisations based on different concentrations of vermicompost leachates were tested, and we found that the lowest concentration can decrease the time to anthesis of *L. anceps* (according to some of our studies), (Figure 5).

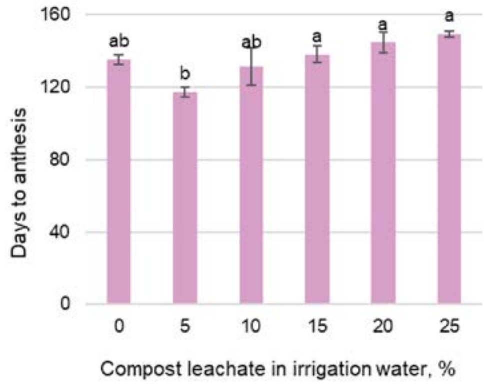


Figure 5. Days to first flower anthesis of *Laelia anceps* with different concentrations of vermicompost leachates. Means ± SD with different letters indicate statistical differences (LSD at 5%).

Several countries are working on their native orchid-flora for ornamental purposes, from the characterization of their plants and blooms to the management of commercial crops and the generation of hybrids, which requires several years of observation and waiting for results but ensuring the intellectual property of their orchid-derived floricultural products. It is also necessary that these products meet the demands of sustainability in production, established by the commercial chain and society (carbon footprint, specialty crops, etc.) Therefore, research on environmental management and inputs will continue to be essential, both for the control of time to flowering and other morpho-floral traits, since flowering behavior is species-dependent. Thus, research for the appropriate use of environmentally friendly inputs, such as biofertilizers, biostimulants, plant growth regulators, environmental management combined with clean energies, among others, must increase to effectively and sustainably complement traditional production systems.

CONCLUSIONS

The long juvenile periods of orchids will continue to be a challenge for both researcher and grower. The data presented in this review show that it is feasible to reduce the time to flowering of ornamental orchids by using different types of environmental controls and inputs, among which nutrition and plant growth regulators are of particular importance. A major challenge is attempting a better understanding of how these factors interact. Although molecular advances aimed at improving commercial flowering traits to overcome the adverse effect of time to flowering, only a few orchid genera and species have been able to achieve this goal. However, further exploration of native species is also essential to increase the diversity of floricultural products in countries with less access to biotechnological advances.

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Patterns of orchid abundance and diversity in an elevation gradient in the Cloud Forest of Tezonapa, Veracruz, Mexico

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ABSTRACT

Objective: To analyze the orchid abundance and diversity in an elevation gradient in the tropical montane cloud forest (TMCF) of Tezonapa, Veracruz, Mexico.

Design/Methodology/Approach: Orchids were sampled in 100×20 m temporary transects, randomly distributed in an elevation gradient (T1=800-900, T2=901-1,000, T3=1,001-1,100, T4=1,101-1,200, and T5=1,201-1,300 m). Each specimen was georeferenced, species were identified, and the conservation status was determined.

Results: The diversity in the area reached 26 orchid species from 16 genera. The passport data of 204 specimens were recorded.

Study Limitations/Implications: The greatest abundance, richness, and diversity were recorded in T3. This result matches the favorable temperature and humidity conditions required for the development of orchids in a TMCF.

Findings/Conclusions: *Stanhopea tigrina* is in danger of extinction. Therefore, the following protocol is urgently required: *in vitro* propagation, release of individuals into the environment, and follow-up of wild populations for genetic improvement purposes.

Key words: Cloud Forest, NOM-059, Orchidaceae, orchid flora.

INTRODUCTION

Family Orchidaceae is composed of approximately 25,000 species and makes up 10% of the world floral diversity (Atwood, 1986). Mexico's diverse physiography and weather conditions have favored many biological families, particularly 167 genera and 1,296 species of family Orchidaceae (Rzendowski, 2006).

Orchid abundance is threatened by weather and anthropic factors that damage, destabilize, and carelessly exploit their populations, ultimately decimating them (Soto-Arenas *et al.*, 2007; Toledo *et al.*, 2020). Despite their environmental, industrial, medicinal, food, religious, and economic importance, at least 22 orchid species have become extinct in Mexico (Emeterio-Lara *et al.*, 2016). Wild orchids are informally sold in local markets or online, which increases the number of threatened specimens that are plundered, according to the NOM-059-SEMARNAT-2010 Mexican standard (Tejeda-Sartorius and Téllez-Velasco, 2017; SEMARNAT, 2010, 2019).

Most orchids are found in tropical ecosystems; however, the tropical montane cloud forest (TMCF) houses 60% of the Mexican orchid flora (Toledo *et al.*, 2020; CONABIO, 2022). The TMCF accounts for 18,534 km^2 (1%) of the Mexican territory and faces disturbance problems resulting from anthropogenic factors, leading to the loss of orchid biodiversity (Francisco-Ventura *et al.*, 2018; Toledo *et al.*, 2020; CONABIO, 2022).

The TMCFs record the highest orchid diversity in Mexico (443 species). Most of them (257 species from 93 genera) are found in the state of Veracruz (Villaseñor, 2010). Few researches have studied the abundance and diversity of orchids in the TMCFs of the state of Veracruz (Tejeda-Sartorius *et al.*, 2013; Tejeda-Sartorius and Téllez-Velasco, 2017). Díaz *et al.* (2012) reported that the main causes of the patterns of diversity and abundance are topography, slope, exposure to solar radiation, and altitude. Consequently, the patterns of orchid abundance and diversity were analyzed in an elevation gradient in the TMCF of Tezonapa, Veracruz, Mexico, to propose potential conservation and management plans (Tejeda-Sartorius and Téllez-Velasco, 2017).

MATERIALS AND METHODS

The work was carried out from July to December 2022, in the municipality of Tezonapa, Veracruz (Figure 1), which has a border to the north with the municipality of Zongolica, to the west with the municipality of Omealca and the state of Oaxaca, to the south with

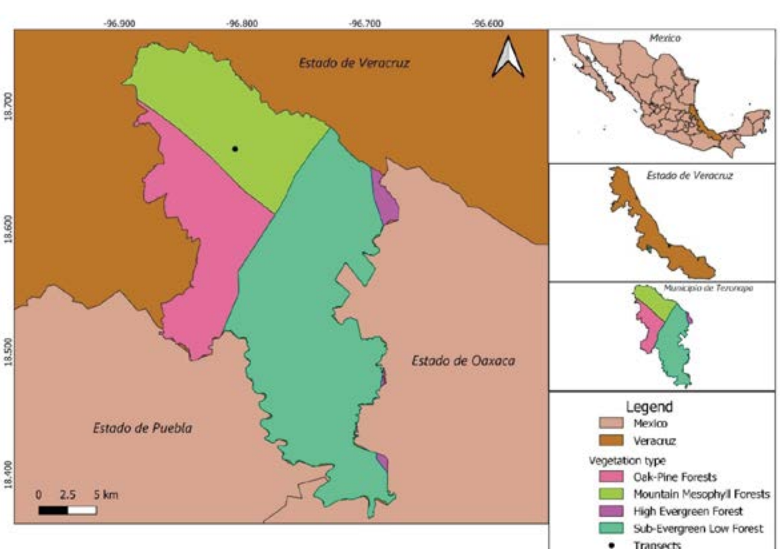

Figure 1. Orchid sampling area in the TMCF of Tezonapa, Veracruz (CONABIO, 2001).

the states of Oaxaca and Puebla, and to the west with the municipality of Zongolica and the state of Puebla. The municipality is located between 18.366° and 18.75° N and 96.666° and 96.916° W, at an altitude between 60 and 1,500 m. The average temperature ranges from 18 to 26 °C, while the mean annual rainfall ranges from 2,400 to 3,100 mm (SIEGVER, 2020; INEGI, 2021). The vegetation includes oak and pine forest, tropical montane cloud forest, montane tropical evergreen forest, and lowland subperennifolious tropical forest; meanwhile, the soils include humic acrisols, orthic acrisols, chromic luvisols, orthic luvisols, chromic vertisols, pellic vertisols, and lithosols (CONABIO, 2001; INIFAP-CONABIO, 1995).

The orchids were collected following the Hágsater-Stewart method (1986) and the sampling was carried out in 100×20 m temporary transects (Mostacedo and Fredericksen, 2000), randomly distributed in an elevation gradient (T1=800-900, T2=901-1,000, T3=1,001-1,100, T4=1,101-1,200, and T5=1,201-1,300 m). Each specimen was labeled with its collection number, transect number, and habitat; the georeferenced coordinates were recorded with a Garmin eTrex® 10 GPS. The collected plants were taken to the nursery of the Instituto Tecnológico de Zongolica for their conservation and taxonomic identification.

The orchids were identified according to Pridgeon *et al.* (2005, 2009), Blanco *et al.* (2007), Soto-Arenas *et al.* (2007), and Chase *et al.* (2015). The data were checked with the information available in virtual herbaria (AMO) and digital platforms (CONABIO, iNaturalista, Missouri Botanical Garden, and Global Biodiversity Information Facility (GBIF)). Additionally, the conservation status was determined according to the NOM-059-SEMARNAT-2010 (SEMARNAT, 2010, 2019).

The richness and abundance of the orchid species recorded at different altitudes were compared with the species cumulative curve analysis. The curves were developed using the iNEXT software, based on the number of individuals and species reported for each sample (Chao *et al.*, 2016). When confidence intervals overlap, there is no significant differences in richness and/or abundance (Cumming *et al.*, 2007).

The species inventory completeness was determined calculating the coverage estimation of the sample, which indicates the ratio of the plant community represented by the collected species. When the sample coverage approaches 100%, the sampling has been completed, considering the collection effort and technologies employed. In addition, the values of the effective number of species (q^0 or abundance, q^1 or abundant species, and q^2 or very rare species) can be therefore compared (Chao and Jost, 2012) with sets whose inventory has a similar completeness level (Magurran and Henderson, 2010). A Principal Component Analysis (PCA) was likewise applied, using the abundance correlation matrix for each classified species for the altitude range. The orchid species were classified based on the classification criteria of epiphytic plants; their frequency was determined with the Infostat software (2021), based on the elevation gradient.

RESULTS AND DISCUSSION

Orchid abundance is made up of 204 specimens, 26 species, and 16 genera. Figure 2 includes some of the most representative species. *Epidendrum* was the genus with the highest number of species (*E. amphistomum*, *E. melistagum*, *E. paranthicum*, *E. radicans*, *E.*

ramosum, and *E. rogidum*), followed by *Campylocenthum* (*C. micranthum* and *C. schiedei*), *Dichaea* (*D. glauca* and *D. muricatoides*), *Isochilus* (*I. unirantiacus* and *I. aurantiacus*), and *Prosthechea* (*P. ochracea* and *P. cochleata*). According to the distribution per habit, the most abundant species are: epiphytes (93%), ground and epiphytes (6%), and lithophytes and epiphytes (1%). Tejeda-Sartorius and Téllez-Velasco (2017) reported a similar diversity in their study of a TMCF in the state of Veracruz; they identified 36 orchid species from 25 genera, the most diverse of which was *Epidendrum* (7). The orchid abundance reported in this study accounts for 5.86% and 10.11% of the orchid flora of Mexico and the state of Veracruz, respectively (Villaseñor and Gual-Díaz, 2014; Villaseñor, 2010).

T3 (1,001-1,100 m, the intermediate elevation stratum) recorded greater abundance and richness than the other strata (Table 1). However, Díaz *et al*. (2012) reported different results: they found greater diversity and abundance of plant species at higher than at lower altitudes. They attributed this result to the intensive management, easy access, and high rates of resource exploitation.

The T3 altitude recorded the highest diversity of species and abundance of individuals per species. Meanwhile, T5 was significantly similar recorded the same number of species, but highest abundance (Figure 3).

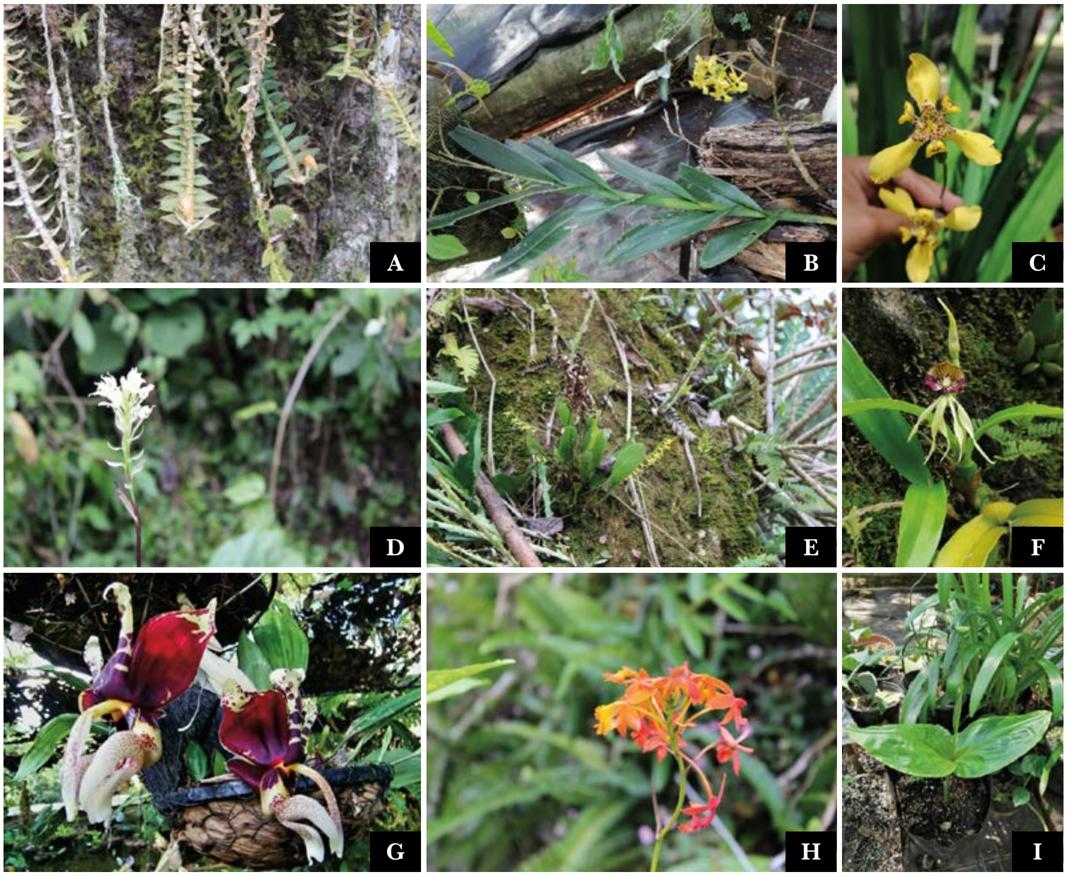

Figure 2. Representative orchid species of the TMCF of Tezonapa, Veracruz. A) *Dichaea muricatoides*, B) *Epidendrum amphistomum*, C) *Trimezia steyermarkii*, D) *Govenia alba*, E) *Stelis platystylis*, F) *Prosthechea cochleata*, G) *Stanhopea tigrina*, H) *Epidendrum radicans*, and I) *Malaxis excavata*.

Table 1. Indicators of the effective number of orchid species at various altitudes in a TMCF.

Indicator	Abundance Species richness Sample coverage				
	T1 (800-900)	**T2 (901-1000)**	**T3 (1001-1100)**	**T4 (1101-1200)**	**T5 (1201-1300)**
Abundance	4	52	61	53	34
Species richness	2	9	14	9	9
Sample coverage	1.0	0.9	0.9	0.9	0.9
q^0	2	9	14	9	9
q^1	1.7	6.6	11	7.3	6.7
q^2	1.6	5.4	9	6.6	5.5

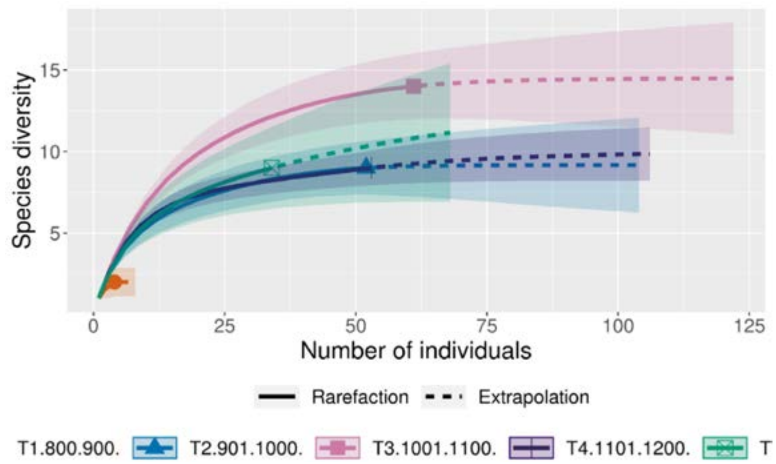

Figure 3. Rarefaction and extrapolation curves of the orchid diversity and number of individuals found at various elevation ranges in the TMCF of Tezonapa, Veracruz, México.

The T3 altitude community had the highest abundance of species and the greatest number of abundant species and rare orchid species, indicating that its diversity is structurally stable (Figure 4). Figure 4 shows that T1 had an unstable structure and a low abundance (two common species and one rare species). Its proximity to human settlements and agricultural and livestock areas fragments the ecosystem and the diversity of orchids. Baltazar and Solano (2020) recorded a q^1 of 7.1 orchid species, attributing this diversity to temperature, humidity, and tree canopy.

The first two PCs show a cumulative variance of 61% (Figure 5), while PC3 has an 82% variance. In this sense, 82% of the orchid species can be found at three altitudes: 14 species at T3 and 9 species each at T2, T3, and T5. The remaining altitudes had a lower variation (*e.g.*, T1 only had two orchid species). Finally, the two species with the greatest variation in T3 were *M. densa* and *L. aurantiacus*.

Stanhopea tigrina is included in NOM-059-SEMARNAT-2010 as a category A species (endangered). Nevertheless, the TMCF in which that species was found is not included among the Federal Protected Natural Areas of Mexico (CONANP, 2023). Therefore, according to Tejeda-Sartorius and Téllez-Velasco (2017), the orchid flora of Veracruz

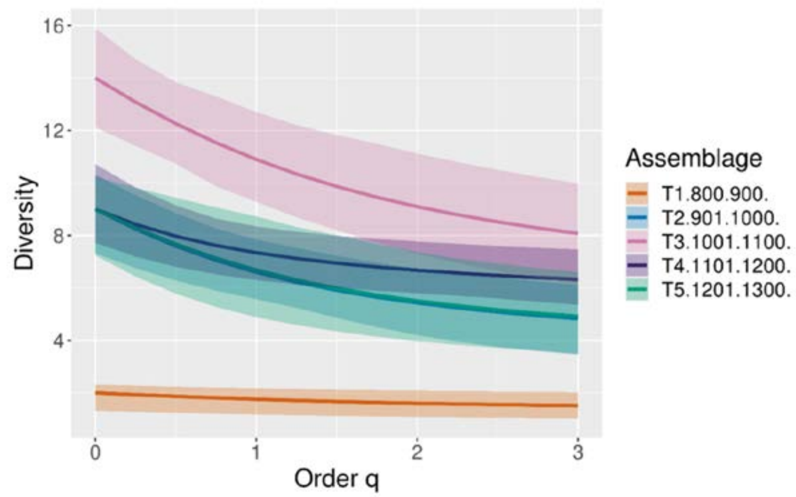

Figure 4. Comparison of the effective number of orchid species of order q^0, q^1, and q^2 of the community in the elevation gradient of the TMCF of Tezonapa, Veracruz, México.

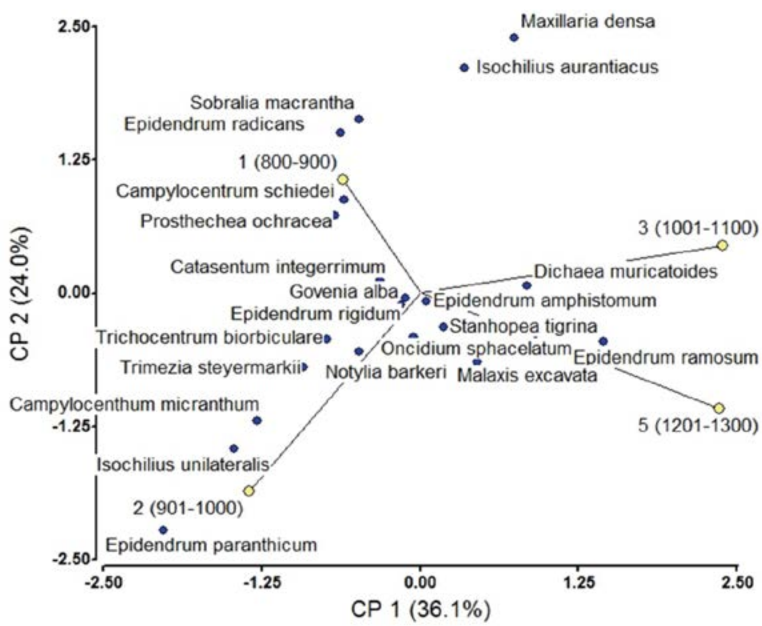

Figure 5. Elevation variation of the diversity of orchid species in the TMCF.

requires further studies to develop public policies for the conservation and management of the TMCF.

CONCLUSIONS

The tropical montane cloud forest of Tezonapa, Veracruz proves the important relationship between this plant community and the orchid flora, recording an abundance of 204 specimens from 26 species. The greatest abundance and diversity were recorded at an altitude of 1,001 to 1,100 m, which favors the development of orchids. *S. tigrina* is an endangered species that requires an urgent protocol that should include *in vitro* propagation, the release of individuals into the environment, and follow-up of wild populations.

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