ISSN: 2594-0252

AGRO PRODUCTIVIDAD

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Año 16 • Volumen 16 • Número 7 • julio, 2023

Colegio de Postgraduados

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Año 16, Volumen 16, Número 7, julio 2023, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 29 de agosto de 2023.

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Sexual propagation of *Abies religiosa* (Kunth) Cham, by immersion in water at different temperatures and periods

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ABSTRACT

Objective: To increase the germination percentage of *Abies religiosa* by immersion in water with different temperatures and periods.

Design/Methodology/Approach: A completely randomized factorial experiment was carried out with five temperatures (10, Room temperature (Rt), 30, 40, and 50 °C) and 13 periods expressed in seconds (10, 20, 40, 60, 600, 1200, 1800, 2400, 3000, 3600, 7200, 14400, and 21600), with a total of 65 treatments and 30 repetitions per treatment. The seeds were dipped in water with the temperatures and periods of each treatment to obtain the highest germination percentage.

Results: The best pre-germination treatment (73% germination) was obtained with the combination of 40 °C and 7,200 s. Meanwhile, the treatments with the lowest percentage (14%) were the combinations 10 °C×20 s and 10 °C×40 s. Twenty-six treatments recorded a >50% germination, reaching a peak value of 8.56 at 11.7 days.

Study Limitations/Implications: Keeping the water temperature and the immersion period constant during the experiment was more difficult in the longer treatments, with the lowest and highest temperatures.

Findings/Conclusions: *Abies religiosa* registers acceptable germination percentages with the highest temperature (50 °C).

Keywords: Germination, temperature, time, peak value.

Citation: Hernandez-Valera, L., Cetina-Alcalá, V. M., Ramírez-Herrera, C., Jasso-Mata, J., Quiroz-Ibáñes, I. F., & González-Rosas, H. (2023). Sexual propagation of *Abies religiosa* (Kunth) Cham, by immersion in water at different temperatures and periods. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2298

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: June 15, 2022.
Accepted: June 18, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 3-10.

INTRODUCTION

Regeneration in conifers is a slow and difficult process that involves sexual reproduction as a propagation alternative under natural conditions. The oyamel (*Abies* sp.) genus reproduces with some ease in the forests, despite its low viability percentages and high percentage of vain seeds (Franklin, 1974). The sexual propagation of trees through seed germination is the main means of obtaining more vigorous, adaptable, and healthy plants

Image by Sr. M. Jutta at Pixabay

(Briscoe, 1990; Trujillo-Navarrete, 1992). Seeds are considered the most important source of germplasm in forest species and they are the most frequently used source for the mass reproduction of plants (Meza, 2009). However, they are limited by the dormancy resulting from internal and external factors, which is only interrupted when adequate germination conditions are present. However, when this phenomenon does not occur or takes place gradually, the germination rate is very low (Rodríguez-Sánchez, 1995).

Several causes determine dormancy, including rudimentary or physiologically immature embryos, mechanically resistant or impermeable seed coats, germination inhibitors, and insufficient storage (Vindas, 2013). Varela and Arana (2011) and other authors consider this as an adaptation that contributes to survival, since it restricts germination in the face of unfavorable environmental factors (*i.e.*, temperature, humidity, and gaseous environment). Several pre-germination treatments can be used to remedy this situation and break seed dormancy. The most common methods are stratification, scarification, and leaching (Donoso, 1993; Franz-Eugen, 1996).

Consequently, several pre-germination treatments were evaluated to increase the germination percentage in *Abies religiosa* through their immersion in water with different temperatures and periods.

MATERIALS AND METHODS

The study was carried out in the Postgrado en Ciencias Forestales of the Colegio de Postgraduados - Campus Montecillo (19° 27’ 38.95” N and 98° 54’ 24.96” W), located at 2,246 m.a.s.l. The seeds used were donated by the San Luis Tlaxialtemalco nursery, located in Xochimilco, Mexico City. The seeds were collected in 2015 at the ejido San Andrés Hueyacatitlán, Puebla, Mexico (19° 15’ 20” N and 99° 47’ W). The experiment was carried out in thirty 20×27 cm plastic trays.

A 5×13 factorial experiment was established according to a completely randomized design, resulting in 65 combinations. The following factors were taken into consideration: temperature (10, room temperature (Rt), 30, 40, and 50 °C) and periods (10, 20, 40, 60, 600, 1200, 1800, 2400, 3000, 3600, 7200, 14400, and 21600 seconds). Each seed was considered as an experimental unit (EU). Thirty repetitions were performed for each combination, reaching a total of 1,950 EUs.

Once the 30 trays were ready, 200 mL of water were added to moisten their protective cloth. The seeds of Abies religiosa were placed in a beaker to which cold or hot drinking water was added, depending on the required temperature, which was measured with a digital thermometer.

Variables

Germination

The seed was considered to have already germinated when the radicle appeared.

Peak Value (PV)

PV is the maximum value obtained by dividing the germination percentage accumulated on each measurement date by the number of days elapsed to date.

Number of days to peak value (NDPV)

NDPV is the number of days necessary to reach a certain germination level, when the accumulated germination divided by the number of days of the test reached the maximum value (Czabator, 1962; Kolotelo *et al.*, 2001). The germinated seeds were evaluated daily during a 30 d period.

Data analysis

To determine which treatments recorded the highest germination percentages —taking into account the temperature and period that the seeds were exposed—, an analysis of variance (ANOVA) and the Tukey-Kramer significant difference test were used. The InfoStat software was also used (InfoStad, 2011).

The experimental design used was the following:

$$Y_{ijk}=\mu+T_i+S_j+TS_{ij}+B_k+\varepsilon_{ijk}$$

Y_{ijk}=observations of the response variable (RV) obtained from the i^{th} temperature (°C) with the j^{th} period, in the k^{th} block; μ=overall mean; T_i=effect of the i^{th} temperature (°C); S_j=effect of the j^{th} period; B_k=effect of the k^{th} block; TS_{ij}=effect of the interaction of the i^{th} temperature with the j^{th} time; ε_{ijk}=experimental error.

RESULTS AND DISCUSSION

Analysis of variance

Based on the ANOVA, significant effects were observed on temperature, as well as on its interaction with the periods applied (Table 1). Likewise, the combination which generated the highest germination percentage was determined.

Temperature effect

The different letters in the Tukey-Kramer test ($v-p$=0.0001) indicated significant differences between the temperatures used in the experiment (Figure 1). Three out of the five temperatures obtained similar means. The 30 °C temperature recorded the best germination percentages, followed by 40 °C (73% germination). The 50 °C temperature registered the lowest value range (23-66%).

Table 1. Significant differences between *Abies religiosa* treatments and interactions were determined with an analysis of variance.

Variable	P-valor		
	Temperature	Time	Temperature × time
Germination Capacity	0.0001	0.3471	0.0111
VP	0.9698	1.0000	1.0000
NDVP	0.9604	1.0000	1.0000

Alpha=0.05. PV (VP)=Peak value. NDPV (NDVP)=Number of days to peak value.

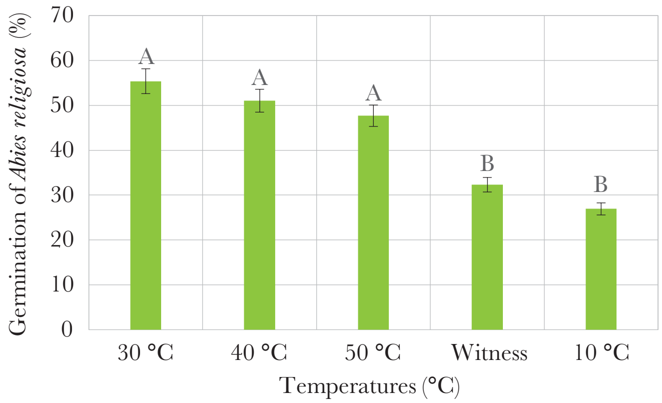

Figure 1. Application of five different temperatures for the reactivation of the dormancy state in *Abies religiosa* seeds.

Effect of the applied periods

According to Figure 2, the statistical analysis and the Tukey–Kramer test showed no significant difference between the periods used to increase the germination of Abies religiosa in this research, regardless of their length.

Effects of temperature × seconds interaction

The analysis determined that there is an interaction between the temperatures and the period that the seed was immersed in water (Table 2). A 73% germination was obtained with the best combination: a temperature of 40 °C and a period of 7,200 s. The lowest percentage (14%) was obtained with the combinations of 10 °C and 20 and 40 seconds. The best treatment must be determined based on the combination of both factors.

Peak value and number of days to reach peak value

The study lasted 30 d, whose peak value (PV) (8.57) was determined (Table 3). It represents the germination speed. For its part, NDPV was 11.7 (Table 4). In the first two weeks, the radicle was mostly observed in days 9, 10, 11, and 12.

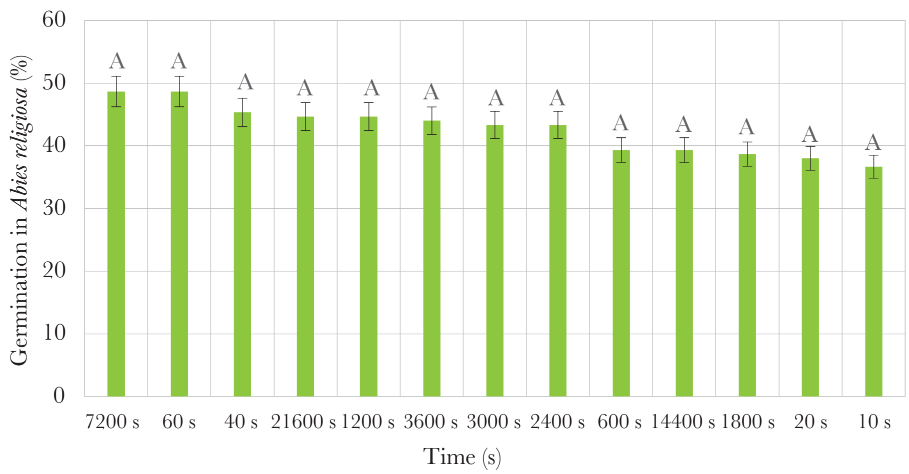

Figure 2. Exposure period of *Abies religiosa* seeds to water. Equal letters indicate equal means.

Table 2. Germination percentage of the different temperature × period combinations in *Abies religiosa* seed.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C
10	17 ab	23 ab	60 ab	48 ab	37 ab
20	19 ab	14 b	57 ab	48 ab	50 ab
40	40 ab	14 b	69 ab	48 ab	52 ab
60	33 ab	40 ab	57 ab	50 ab	66 ab
600	17 ab	17 ab	60 ab	53 ab	48 ab
1200	28 ab	23 ab	57 ab	57 ab	60 ab
1800	37 ab	20 ab	47 ab	43 ab	47 ab
2400	50 ab	23 ab	67 ab	40 ab	37 ab
3000	28 ab	27 ab	52 ab	52 ab	61 ab
3600	37 ab	41 ab	45 ab	60 ab	37 ab
7200	20 ab	37 ab	63 ab	73 a	50 ab
1400	47 ab	31 ab	30 ab	40 ab	53 ab
21600	53 ab	40 ab	57 ab	50 ab	23 ab

Rt=Room temperature.

The results showed different germination percentages for *Abies religiosa*. The highest value (73%) was higher than the percentage reported by Manzanilla (1974), who obtained a 45-49% germination with recently collected seeds without any kind of treatment. In contrast, a 96.8% germination was obtained when the seeds were soaked in coconut water for seven days. This has been the most successful pre-germination treatment for the *Abies religiosa* species (Mayen, 1987), followed by stratification at 1 to 5 °C for 14 to 28 d, which obtained a 70% germination (Patiño, P. Garza, Y. Villagomez, I. Talavera, and F.

Table 3. Peak germination value of *Abies religiosa* seeds.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C	Average
10	8.81 a	8.44 a	8.59 a	8.66 a	8.43 a	8.58 a
20	8.71 a	8.52 a	8.56 a	8.49 a	8.35 a	8.50 a
40	8. 70 a	8.81 a	8.59 a	8.39 a	8.48 a	8.56 a
60	8,76 a	8.53 a	8.51 a	8.50 a	8.43 a	8.53 a
600	8.78 a	9.04 a	8.51 a	8.58 a	8.42 a	8.57 a
1200	8.52 a	8.96 a	8.47 a	8.42 a	8.35 a	8.48 a
1800	8.47 a	8.86 a	8.33 a	8.47 a	8.35 a	8.45 a
2400	8.59 a	8.80 a	8.49 a	8.72 a	8.23 a	8.54 a
3000	8.68 a	8.33 a	8.59 a	8.57 a	8.86 a	8.65 a
3600	8.48 a	8.59 a	8.39 a	8.47 a	9.23 a	8.60 a
7200	8.79 a	8.53 a	8.45 a	8.41 a	8.54 a	8.50 a
1400	8.72 a	8.70 a	8.41 a	8.39 a	8.47 a	8.53 a
21600	8.62 a	8.80 a	8.38 a	8.42 a	8.67 a	8.55 a
Promedio	8.64 a	8.67 a	8.49 a	8.49 a	8.51 a	8.56 a

Rt = Room temperature.

Table 4. Number of days in which *Abies religiosa* seeds reached their peak germination value.

Time (seconds)	Rt	10 °C	30 °C	40 °C	50 °C	Average
10	11.40 a	11.85 a	11.66 a	11.57 a	11.90 a	11.69 a
20	11.50 a	11.75 a	11.70 a	11.80 a	12.00 a	11.78 a
40	11.53 a	11.40 a	11.66 a	11.92 a	11.80 a	11.70 a
60	11.50 a	11.75 a	11.76 a	11.80 a	11.89 a	11.76 a
600	11.40 a	11.20 a	11.77 a	11.68 a	11.93 a	11.71 a
1200	11.75 a	11.28 a	11.82 a	11.88 a	12.00 a	11.82 a
1800	11.81 a	11.33 a	12.00 a	11.84 a	12.00 a	11.86 a
2400	11.66 a	11.42 a	11.80 a	11.50 a	12.18 a	11.73 a
3000	11.62 a	12.00 a	11.66 a	11.68 a	11.47 a	11.64 a
3600	11.81 a	11.66 a	11.92 a	11.83 a	11.09 a	11.69 a
7200	11.50 a	11.72 a	11.84 a	11.90 a	11.80 a	11.80 a
1400	11.53 a	11.55 a	11.88 a	11.91 a	11.81 a	11.74 a
21600	11.62 a	11.41 a	11.94 a	11.93 a	11.57 a	11.73 a
Promedio	11.61 a	11.58 a	11.79 a	11.79 a	11.81 a	11.74 a

Rt=Room temperature.

Camacho, 1983; Willian, 1985). However, the most recent pre-germination treatments failed to exceed the 70% germination rate proposed by Patiño *et al.* (1983) and Willian (1985).

Authors such as Iglesias-Andreu *et al.* (2010) subjected *Abies religiosa* to ionizing radiation or gamma radiation, obtaining 62% germination with the application of a 5-Gy dose. Other authors, including Zulueta-Rodríguez, L.G. Hernández-Montiel, and Ruiz-Ramirez (2015) experimented with hydro and bio-conditioning to reactivate the embryo, through hydro-conditioning of the seeds in water, oxidation through bubble flow, and imbibition in a bacterial suspension, obtaining a 49-70% germination with a 12-h hydro-conditioning.

The germination rate of *Abies religiosa* is lower (15%) than the rate for the fresh seeds of *Abies guatemalensis*. It also decreases with storage: 2% reductions were recorded when the seeds were stored for one year at 3-5 °C temperatures (Donahue, Dvorak, Gutierrez, and Kane, 1985; Dvorak and Donahue, 1992). De Pascual-Pola, Musálem, and Ortega-Alcalá (2003) mention that large, heavy cones with abundant seeds have low viability and are equivalent to lighter, smaller cones, with fewer seeds but with a higher germination capacity.

Montserrat-Arista, Talavera, and Herrera (1992) mention that 33% of the larger seeds of *Abies pinsapo* Boiss germinate, in contrast with 8 to 26% of all the smaller seeds as a whole (Monserrat Arista, 1993).

Ortiz-Bibian *et al.* (2019) indicate that the variation between populations influences the germination of *Abies religiosa*. The seeds of populations that grow from 3,000 to 3,500 m.a.s.l. have the highest viability and germination values (48.7% and 19.6%, respectively), between 12 and 14 days. Likewise, viability and germination vary according to the area where the cones were collected; for example, Rio Frio, Ixtapaluca, Mexico registered a 37.33% viability and a 11.6 d germination for *Abies religiosa* seeds (de Pascual-Pola *et al.*, 2003). Pascual-Pola *et al*. (2003) likewise report 44.69 to 61.78% of non-germinated seeds. Franklin (1974) reported 35 to 93% non-viable seeds for *Abies amabilis* (Dougl.) Forb., *A. concolor* (Gord.) Engelm., and *A. magnifica*. A. Murr. The presence of non-viable seeds is considered a characteristic of the genus *Abies* and is associated with various causes, including an incorrect procedure for the collection and handling of cones and seeds, genetic irregularities, environmental adversities, pollen infertility, and entomological damage (Bramlett *et al*., 1977; Franklin, 1974; Schopmeyer, 1974). Other causes include inbreeding and prezygotic events, including ovule abortion, parthenocarpy or the absence of pollination or fertilization. The resulting empty and wrinkled seeds have a thin or shrunken embryo, if any at all (Hartmann and Kester, 1988). This phenomenon is caused by incompatibility in the passage of pollen, the stunted growth of the pollen tube, or fertilization irregularities. This problem would be explained by postzygotic events, such as ovule abortion during postfertilization, the partial formation of embryos, or their early abortion, which is related to development problems (Baskin and Baskin, 2014).

Even under ideal conditions, storage also has negative effects on germination, as a result of aging and deterioration (Shaban, 2013). This could be one of the main causes of a low germination percentage, since the seed was stored for five years (2015-2020), before it was germinated.

CONCLUSIONS

The *Abies religiosa* seed was able to germinate and withstand a temperature of 50 °C in the longest periods. It was one of the three temperatures that generated the highest germination percentages (26 of the 65 treatments generated). Eight of the best germination percentages (50-73%) were obtained during the shortest period in which the seeds were immersed in water (10). These are the most accurate and their use is recommended, because the temperatures and periods in which the seeds should be removed and placed in the trays were controlled with greater efficiency.

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Colegio de Postgraduados

Estimates to determining the agricultural tractor fleet in Mexico

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ABSTRACT
Objective: the objective of the study was to know the current state of the national fleet of agricultural machinery.
Design/Methodology/Approach: an estimate of the tractor fleet was made by adding the number of tractors with one year of lag, plus the number of tractors from national sales, minus the number of tractors leaving the fleet due to the end of their useful life, the period from 2007 to 2027.
Results: there is a downward trend in the tractor fleet in the period 2007 to 2027. If the downward trend continues, the tractor fleet will have decreased by 114 thousand units in 2027, compared to the 238 thousand existing in 2007. This means a drop of 52%, compared to the initial year, which would leave the fleet of tractors with 124 thousand units.
Limitations/Implications of the study: the main limitation is the non-existence of official data after 2007. Therefore, in order to know the current state of the national fleet of tractors in Mexico, estimates such as the one carried out in this investigation are required.
Findings/Conclusions: The estimate of the tractor industrial fleet in Mexico indicates that annually the number of tractors that are added is less than the number of obsolete tractors that leave the fleet, which indicates that the rate of mechanization will decrease in the future. To avoid such a trend, the Government is required to support investments in agricultural machinery.

Keywords: farm machinery fleet, mechanization index, tractor, Mexico.

Citation: Salas-Gutiérrez, J. C., Mora-Flores, J. S., García-Salazar ,J. A., & Garduño-García, Á. (2023). Estimates to determining the agricultural tractor fleet in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2360

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: August 15, 2022.
Accepted: June 17, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 11-19.

INTRODUCTION

Mexico is a country with a land area of 1.96 million km^2, of which 20.6 million ha were used for agricultural land, and more than 550 agricultural products are obtained in different seasons and periods (SIAP, 2019). Agriculture in Mexico is mainly divided into two types of production, traditional and intensive, marked mainly by social and economic differences. Traditional agriculture is generally rainfed and is located, in most cases, in the southern states of Mexico. It is basically for subsistence and is the main source of income for small producers, who do not have the capacity to buy technology or to install infrastructure. Just as they do not have techniques or training for sowing; they only have ancestral ways to cultivate the land that result in low productivity (CEDRSSA, 2017).


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Intensive agriculture, generally irrigated, are large plantations of agricultural products, carried out by agricultural companies whose objective is the commercial production of large volumes. It is generally found in the northern states of Mexico, it uses high-tech infrastructure such as irrigation systems, tractors, modified seeds, as well as hired and trained personnel, since it is more linked to national and international markets (CEDRSSA, 2017).

The evolution of agricultural machinery in the 20^{th} century has been so spectacular that, of the three advances made throughout the history of agricultural machinery, two of them can be considered to mark the beginning and end of the 20^{th} century. The first fundamental advance was recorded when man, who was removing the earth by hitting it with a hoe-type tool, decided to advance with it inserted into the ground to overcome the pulling force. This is how the plow was born at an indeterminate time in prehistory. The construction of the first tractor with an internal combustion engine, due to Froelich in 1892, marks the beginning of the current tractorization. In recent times, electronic and computer devices have been used in machines, which measure diverse variables related to the work they perform, keep the information in records and even decide how the machine should be commanded (Ruiz-Altisent *et al.*, 2000).

The tractor has been an icon of technological change in agriculture, its adoption within the farm has meant various combinations, relationships and established forms of production in a locality, region and country. The dynamics of the tractorization process is defined by the balance given by the units that enter the fleet and by those that are withdrawn due to the end of their useful life (Muñoz *et al.*, 2012).

The farm tractor fleet of a region or country is sometimes taken as an important indicator of investment in agriculture (Muñoz *et al.*, 2011).

The demand for tractors depends, in general terms, on the agricultural activity that takes place in a certain region, a fleet and a greater demand are placed in those agricultural areas where the intervention of man is greater to create favorable conditions to carry out this activity (Mora-Flores, 1986). The level of mechanization of farms is a factor to consider in any technical or economic analysis of the sector, so it is vitally important to have as accurate and detailed knowledge as possible of the national fleet of agricultural tractors (Ministry of Agriculture, Fisheries and Food, no date).

One of the ways to estimate the degree of mechanization of a region or country is through the number of tractors and the intensity of tractorization, measured by power per surface unit (Muñoz *et al.*, 2011).

The inventory of agricultural machinery in Mexico was estimated for the first time in the 1930 Agricultural and Livestock Census, which indicated that there were 3.8 thousand tractors and 8.5 thousand locomobiles in the nation (INEGI, 1936). For 1940, the Livestock Agricultural Census registered 4549 units of agricultural tractors. In 1950 there was a record of 22 711 (INEGI, 1959). By 1982, there were 157 964 tractors (Negrete *et al.*, 2013). The VII National Agricultural Census of 1991 indicated that there were 177 000 tractors and by 2007, according to the VIII Agricultural, Livestock, and Forestry Census, there were 238 000 tractors, of which only 227 000 were in operation. The evolution of the machinery fleet in Mexico had a great growth from 1930 to 1991 according to data from the Food and Agriculture Organization of the United Nations (FAO, 2021). In 1991 there were 317 thousand units, the highest number ever recorded in the country. Later, in 2007 the number decreased by 79 thousand units, 25% in percentage terms, with a tendency of decrease by almost 5 thousand units per year. In the years after 2007 there is no official record that considers the total number of tractors registered in the country.

According to data from INEGI (2019) reported in the 2019 National Agricultural Survey, of the 100% of the production units that own machinery and equipment to carry out agricultural activities, 65.8% use tractors, 36.4% planters, 28.7% plows, 27.3% disc harrows, 12.1% cultivators for tractors, 6.6% threshers and 0.22% motorized cranes. This is an overview that the tractor is one of the most important elements used in agriculture. One of the most important studies on the number of agricultural tractors in Mexico was conducted by Negrete *et al.* (2013), who made an analysis of the farm tractor fleet in Mexico and estimated 224 thousand tractor units in 2011. Hernández-Ávila *et al.* (2022), in an evaluation of the level of techno-agricultural mechanization in six municipalities of the Toluca Valley, established in their conclusions that the tractor fleet has been in use for more than 15 years, so it is necessary to promote a renewal plan.

Considering the importance of agricultural tractors as one of the most important means of production in the agricultural sector, this study aimed to know the current state of the national fleet of agricultural machinery in Mexico. It is worth mentioning that there are no official data after 2007 indicating the number of active tractors in the country.

MATERIALS AND METHODS

The geographical scope of the study considers the national territory. Due to the scarce statistics on the number of tractors in the country, an estimate of the tractor fleet was made in a time series from 2007 to 2027. The data presented in the VIII Agricultural, Livestock and Forestry Census 2007 correspond to the base year, which presented a scenario of the real existence of tractors in Mexico. This Census reported the total inventory of 238.2 thousand units of agricultural tractors, of which 177.9 thousand had more than 5 years of use and 54.4 thousand had less than 5 years of use. Of the total inventory, only 227.3 thousand were in operation.

To achieve the objectives, the method of Muñoz *et al.* (2011) cited by Negrete *et al.* (2013) was used. This methodology made it possible to estimate the fleet of tractors at a given time, based on the data reported from the last national census of 2007 and with the consideration of the national market sales reported by the manufacturer John Deere from 2008 to 2021.

To estimate the fleet of tractors, the following expression was used.

$$P_t^T = P_{t-1}^T + T_t^N - T_t^R \tag{1}$$

where P_t^T is the fleet of tractors in year t, expressed in units of tractors; P_{t-1}^T is the fleet of tractors with a one-year lag $t-1$, expressed in units; T_t^N is the number of tractors added to the fleet in year t, expressed in y units; T_t^R is the number of tractors leaving the fleet in year t, expressed in units.

The information to estimate the fleet of tractors was obtained from the sources mentioned below. The number of tractors in year $t-1$ was gotten from the VIII Agricultural, Livestock and Forestry Census 2007 (INEGI, 2009). The number of tractors that are added to the tractor fleet were the sales registered in Mexico from the database published by the manufacturer John Deere (John Deere, 2021). The projection of tractors added from 2022 to 2027 was estimated based on the sales of the previous five years. With the data from John Deere (2021), the number of tractors added to the park in year $t-1$ $\left(T_t^N\right)$ was estimated because there is no official, revised and updated record of the number of new agricultural tractors.

According to Muñoz *et al.* (2011), the number of tractors leaving the park (obsolete tractors) can be estimated knowing the average useful life of the tractor, but the difficulty in obtaining this variable lies in the fact that different final figures will be obtained depending on the selected useful life. Muñoz *et al.* (2012) estimated that the estimated average useful life for an agricultural tractor at the aggregate level is 22 years, depending on whether the tractor comes from a certain group of countries.

For the study, the number of tractors leaving the park at time t was obtained from the difference of the number of tractors at time t minus the number at time $t-1$, and the amount added at time t is added, this variable is expressed in units of tractors.

To calculate the number of obsolete tractors, the following expression by Muñoz *et al.* (2011) cited by Negrete *et al.* (2013) and obtained from equation (1) was used:

$$T_t^R = P_{t-1}^T + T_t^N - P_t^T \tag{2}$$

where T_t^R is the number of tractors leaving the fleet in year t, expressed in units; P_{t-1}^T is the tractor fleet with a lag year $t-1$, expressed in units; T_t^N is the number of tractors added to the fleet in year t, expressed in units; P_t^T is the tractor fleet in year t, expressed in tractor units.

The data was processed with Microsoft Excel (2010), with which the values presented in Table 1 were obtained.

The tractor is a symbol of mechanization of agriculture, hence its great importance; it is the main point of reference to measure mechanization indices in the field (Regalado-Negrete, 2006). To calculate the mechanization index, one of the ways is the cultivated area covered in relation to the number of tractors. For this, the formula indicated by Regalado-Negrete (2006) was used. This index shows the intensity of the use given to tractors to implement agricultural operations.

To calculate the mechanization index, the following formula was used:

$$IMi = \frac{SUPi}{TRACTi} \tag{3}$$

where *IMi* is the mechanization index in state i; *SUPi* is the cultivated area in hectares in state *i* and *TRACTi* is the number of tractors in state *i*.

RESULTS AND DISCUSSION

Table 1 presents the results obtained, showing a downward trend in the machinery fleet from 2007 to 2027. From 2008 to 2010 the average decrease is 4 thousand units, similar to that reported by FAO in the 1991-2007 period, 4.9 thousand units. The largest decrease was observed in 2013 and 2020 with around 8 thousand units lost.

If the downward trend continues, the estimate indicates that in 2027, the tractor fleet will have decreased, by 114 000 units, up to 124 000 remaining units (Table 1).

Regarding the tractor fleet, one of the most important studies is the one carried out by Negrete *et al.* (2013), who estimated a quantity of 223 thousand tractors in 2011 and

Table 1. Estimate of the machinery fleet in Mexico, 2007-2027.

Year	Base year	Obsolete	Sales	Park	Annual change
	P_{t-1}^{T}	T_t^R	T_t^N	P_t^T	
2007	238,830				
2008		17,061	15,640	237,409	1,421
2009		17,061	12,985	233,333	4,076
2010		17,061	10,223	226,495	6,838
2011		17,061	10,140	219,574	6,921
2012		17,061	10,630	213,143	6,431
2013		17,061	8,546	204,628	8,515
2014		17,061	9,896	197,463	7,165
2015		17,061	12,899	193,301	4,162
2016		17,061	13,380	189,620	3,681
2017		17,061	14,778	187,337	2,283
2018		17,061	14,401	184,677	2,660
2019		17,061	10,506	178,122	6,555
2020		17,061	8,737	169,798	8,324
2021		17,061	9,613	162,350	7,448
2022		17,061	11,607	156,896	5,454
2023		17,061	10,973	150,808	6,088
2024		17,061	10,287	144,034	6,774
2025		17,061	10,243	137,216	6,818
2026		17,061	10,545	130,700	6,516
2027		17,061	10,731	124,370	6,330
Average 2007-2009		-	-	236,524	-
Average 2025-2027		-	-	130,762	-
GR		-	-	−44.7	-
AAGR		-	-	−3.2	-

GR=Growth rate in the period 2007/09-25/27; AAGR=Average annual growth rate in the period 2007/09-25/27. Source: elaborated by the authors, based on data from INEGI (2009) and John Deere (2021).

extrapolated for 2015, 200 thousand units with the FAO trend model. In this regard, in this research for the same years, the park estimate was 219 thousand units in 2011 (a difference less than 2%) and 193 thousand units in 2015 (a difference of 7 thousand) compared to the above-mentioned report (Negrete *et al.*, 2013).

According to the information reported in Table 1, estimated national sales from 2008 to 2021 were 162.3 thousand units of tractors, an annual average of 11.6 thousand. The year with the highest sales was 2008, 15.6 thousand units; the lowest years have been 2013 and 2020 with 8.5 and 8.7 thousand units sold. The CoVID-19 health crisis decreased sales in 2020 and severely affected the domestic market for agricultural machinery.

The above results indicate that sales have remained stable; these data are similar to those reported by Palacios *et al.* (2003) cited by Ayala *et al.* (2013), who indicated that since 1997 the Mexican market has been very stable in its sales reports, averaging between 10 and 11 thousand tractors per year.

During the period 2007/09-25/27, the growth rate of the tractor fleet was −44.7%, and the average annual growth in the same period was −3.24% in percentage terms, which implies that the tractor fleet has a negative trend in the analysis period.

Table 2 presents a scenario related to the fleet of agricultural machinery by state. It is observed that eight states have the largest number of tractors, Coahuila, Durango, Guanajuato, Michoacán, Jalisco, Sinaloa, Tamaulipas and Zacatecas that altogether have 149 thousand units, which represents 63% of the national fleet.

Considering the number of tractors and the mechanized area per hectare, an estimate of the mechanization index was made. The results show that some states in the Center and North of the country, such as Guanajuato, Zacatecas, Sinaloa, Tamaulipas, Aguascalientes, Baja California and Durango are the states with the highest rate of mechanization with an average very close to 50 ha per tractor. According to Hernández *et al.* (2022), the previous data is the ideal according to what was proposed by the FAO (50 ha per tractor). Some states in the Southeast of Mexico such as Quintana Roo, Yucatán, Oaxaca and Campeche have a rate of mechanization that exceeds 130 ha per tractor. The previous results are similar to those reported by Regalado-Negrete (2006), 70.8 ha per tractor for the northern zone; 221.6 ha per tractor for the southern zone; and 101 ha per tractor nationwide, in a mechanized area of 18.6 million ha.

With a mechanized area of 15.7 million ha in 2019, there is an average national mechanization index of 114 ha per tractor for an index greater than 10% in this study, compared to the national results presented by Regalado-Negrete (2006). The further scenario for 2027 is even more discouraging, because due to 52% decrease in the tractor fleet compared to the base year causes the mechanization index to increase to 219 ha per tractor; this is far from the recommended optimum of 50 ha per tractor.

Gutierrez-Rodriguez *et al.* (2018) made a diagnosis of tractors and agricultural machinery in the municipality of Atlacomulco, State of Mexico, their results showed that in the P. P. Atlacomulco region there are 8.04 tractors per 100 ha, which is equivalent to a mechanization index of 0.08 ha by tractor. This value reflects that there are big differences among the different production areas in the country; there are highly technical areas with agricultural machinery and areas with deficiencies in machinery.

Table 2. Mechanization Index in the agricultural sector of Mexico by state, 2019.

State	Total area	Tractors	Mechanized area 2019	MI 2019	Tractors	MI 2027
	Thousands of ha	Units 2007	Thousands of ha	tractors/ ha	Units 2027	tractors/ ha
Aguascalientes	128	3,922	124	32	2,047	61
Baja California Norte	180	4,753	177	37	2,481	71
Baja California Sur	41	1,344	40	30	702	57
Campeche	340	2,052	271	132	1,071	253
Chiapas	1,360	3,710	278	75	1,937	144
Chihuahua	1,036	1,561	1,033	662	815	1,267
CDMX	16	3,180	14	4	1,660	9
Coahuila	252	26,749	228	9	13,963	16
Colima	162	294	130	442	153	847
Durango	576	13,447	557	41	7,020	79
Guanajuato	948	21,572	876	41	11,261	78
Guerrero	902	1,400	466	333	731	638
Hidalgo	529	5,363	368	69	2,800	131
Jalisco	1,650	19,907	1,517	76	10,392	146
México	747	8,479	643	76	4,426	145
Michoacán	1,119	13,446	1,004	75	7,019	143
Morelos	137	1,947	124	64	1,016	122
Nayarit	370	4,693	265	57	2,450	108
Nuevo León	330	4,479	330	74	2,338	141
Oaxaca	1,254	3,117	613	197	1,627	377
Puebla	939	6,032	741	123	3,149	235
Querétaro	137	2,496	136	54	1,303	104
Quintana Roo	118	456	70	153	238	294
San Luis Potosí	638	7,347	570	78	3,835	149
Sinaloa	1,059	17,522	1,050	60	9,147	115
Sonora	603	8,705	600	69	4,544	132
Tabasco	266	1,010	109	108	527	207
Tamaulipas	1,326	12,472	1,223	98	6,511	188
Tlaxcala	235	2,765	220	80	1,443	153
Veracruz	1,515	9,396	912	97	4,905	186
Yucatán	699	184	31	170	96	325
Zacatecas	1,051	24,448	1,045	43	12,762	82
National	20,665	238,248	15,764	114	124,370	219

MI=Mechanization Index.Source: elaborated by the authors, based on with data from INEGI (2009) and SIAP (2019).

The lack of resources for the acquisition of agricultural machinery and the ignorance of the reality of the problem of the machinery sector, constitute one of the reasons for the lag in terms of the development of agricultural mechanization. The tractors offered in Mexico are too expensive for small farmers who traditionally practice agriculture; this coupled with the lack of economic resources in cash for the acquisition of new equipment, whose price ranges from 375 to 800 thousand pesos (Ayala-Garay *et al.*, 2012).

In order to reverse the downward trend, in the fleet of agricultural tractors and agricultural machinery in general, it is necessary to encourage an attractive offer for farmers through the granting of credits for the purchase of agricultural machinery. Although there has been support for mechanization, such as the 22 000 tractors granted through the Federal Government from 2013 to 2018 (SAGARPA, 2018) as support for field mechanization, this support is not sufficient for all the agricultural lands in the country. It is necessary to implement better policies focused on the mechanization of the field that help reverse the trend. In order to make it possible that tractors are added to the fleet in greater quantity than the number of tractors ending their useful life.

CONCLUSIONS

The data estimated in this research indicated that the fleet of agricultural tractors in Mexico shows a tendency to decrease. The results of the estimates showed that in the period 2007 to 2027 the number of tractors added to the fleet is notoriously lower than the number of units that leave the fleet. which creates a deficit and a decrease of 114 thousand units in the period.

Estimates indicate that the mechanization index also shows a negative trend. Regarding the mechanized surface in 2019, the index was 114 ha per tractor. The estimate in this research indicates that by 2027 it shall be located at 219 ha per tractor.

The downward trend in the number of tractors makes it necessary to implement credit policies focused on increasing the number of agricultural tractors in the country. Only in this way can the trend in the mechanization index be reversed. Efforts should be directed towards bringing this decreasing index closer to the value recommended by international organizations, which is 50 ha per tractor.

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Colegio de Postgraduados

Morphometry of *Anolis tropidonotus* (Peters 1863, Squamata: Dactyloidae) populations from contrasting habitats

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ABSTRACT

Objective: To evaluate variations in shape and size of two populations of *A. tropidonotus* from two habitats with contrasting vegetation and environmental characteristics.

Design/Methodology/Approach: Twenty-six *A. tropidonotus* specimens were collected and photographed using the TpsDig2 software. Subsequently, three type-I and eight type-II landmarks and nine semi-landmarks were placed. The landmark configuration patterns were evaluated using generalized procrustean and principal component analyses to detect microvariations in individuals of both populations.

Results: The results show intra- and inter-population geometric morphometric variations in *A. tropidonotus*.

Study Limitations/Implications: The geometric morphometric variations recorded in *A. tropidonotus* populations may be caused by biological barriers, clinal variations within the same habitat, competition for territory, different reproductive behaviors in part of the population, and reproductive and physical barriers that generate differentiation between and within *A. tropidonotus* populations.

Findings/Conclusions: The morphometric traits of *A. tropidonotus* showed a wide diversity of shapes. Geometric morphometry was used to differentiate various ecotypes in the evaluated populations.

Keywords: Lacertilidae, homologous landmarks, landmarks, TpsDig, principal component analysis.

Citation: Serna-Lagunes, R., Torres-Cantú, G. B., Llarena-Hernández, R. C., Mora-Collado, N., Andrés-Meza, P., García-Martínez, M. Á., Hernández-Salinas, G., & Salazar-Ortiz, J. (2023). Morphometry of *Anolis tropidonotus* (Peters 1863, Squamata: Dactyloidae) populations from contrasting habitats. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2390

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: October 04, 2022.
Accepted: May 18, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 21-28.

INTRODUCTION

Geometric morphometry is a biostatistical technique commonly used to distinguish, classify, and group individuals from a given population based on specific traits (Zelditch *et al.*, 2012).This technique has been used to study size and shape variations between individuals of one or more populations and even between species of the Squamata order (Meik *et al.*, 2020). The observed morphometric traits can be correlated with the environmental characteristics of the habitat to identify morphological adaptations to the ecosystem (Adams *et al.*, 2004).

Image by Jared Caraway at Pixabay

Within the Squamata order (lizards), the Dactyloidae family is a biologically diverse group in constant evolution. Its species serve as biological models for the development of geometric morphometry studies (Tinius and Russell, 2014; Manthey *et al.*, 2016). Specifically, the *Anolis* genus comprises more than 400 species, some of which inhabit islands and others the landmass of the American continent, from the United States to Brazil (Velasco *et al.*, 2016). The species of the *Anolis* genus are distributed from sea level to 2,000 m.a.s.l., and they can be found in most mainland and island ecosystems, which shows their capacity to adapt to the environmental conditions of their habitats (Stroud and Losos, 2020).

Fifty-five *Anolis* species have been recorded in Mexico. They have adaptive flexibility to tropical environments; their habitat is associated with shrub and tree vegetation close to bodies of water; they can be diurnal or nocturnal, feed on invertebrates, and their morphological trait changes as a result of selection and evolution pressures (Köhler, 2008; Flores and García, 2014; Köhler *et al.*, 2014).

No studies have been conducted about the expected changes in the shape and size of individuals of the *A. tropidonotus* species —commonly known as abaniquillo escamoso mayor or greater-scaled anole, which is native to Mexico, Belize, Guatemala, El Salvador, Honduras, and Nicaragua (Peters, 1863)—, according to the habitat where their populations develop. Likewise, no studies have taken geometric morphometry as the basis for detecting microvariations associated with the type of habitat of this species (Williams, 1983). This study describes variations in the geometric morphometric traits of individuals from two populations of *A. tropidonotus*: one from the tropical evergreen forest and the other from the tropical deciduous forest in central Veracruz, Mexico.

MATERIALS AND METHODS

Study area

The study was carried out in two locations in the central area of Veracruz, Mexico. The first location is the Fortín de las Flores ravine, Veracruz (18.892528, and 97.012), located at an altitude of 892 to 1,010 m.a.s.l., which has a warm temperate climate and evergreen tropical forest vegetation. The second location is Cerro de Lourdes, municipality of Amatlán de los Reyes, Veracruz (18.846811, −96.887268), located at an altitude of 640 to 840 m.a.s.l., with a mainly warm-humid climate and the presence of deciduous tropical forest (INEGI, 2009; Rivera, 2015).

Specimen collection

Between October and November 2019, five collection trips were conducted in each location, from 8 a.m. to noon, through transects, trails, and paths, searching for *A. tropidonotus* specimens. The lizards were caught with direct and manual techniques using elastic bands. The specimens were sacrificed by freezing or alcohol puncture and preserved in 90% ethanol in bottles labeled with information on the collection sites. Subsequently, the specimens were taken to the laboratory, where they were photographed and subjected to a geometric morphometry analysis. The specimen collection was authorized by SEMARNAT (Scientific Collection License no. SGPA/DGVS/001894/18).

Photo session of collected specimens

A millimeter paper was used to indicate where to position most of the animals' bodies. First, the organisms were placed on the millimeter paper and then the area was demarcated based on three parameters, to avoid positioning the samples in different places: 1) the edge of the muzzle; 2) the sides; and 3) the product of the division of the lateral perimeter of the animal (Figure 1a). The photographs were taken with a Canon Rebel T6 camera using an 18-mm lens. An angle limit was established to ensure that all the photographs covered the same space (Figure 1b). The specimens were placed in a standard posture to avoid bias during the extraction of the pure form (Figure 1c).

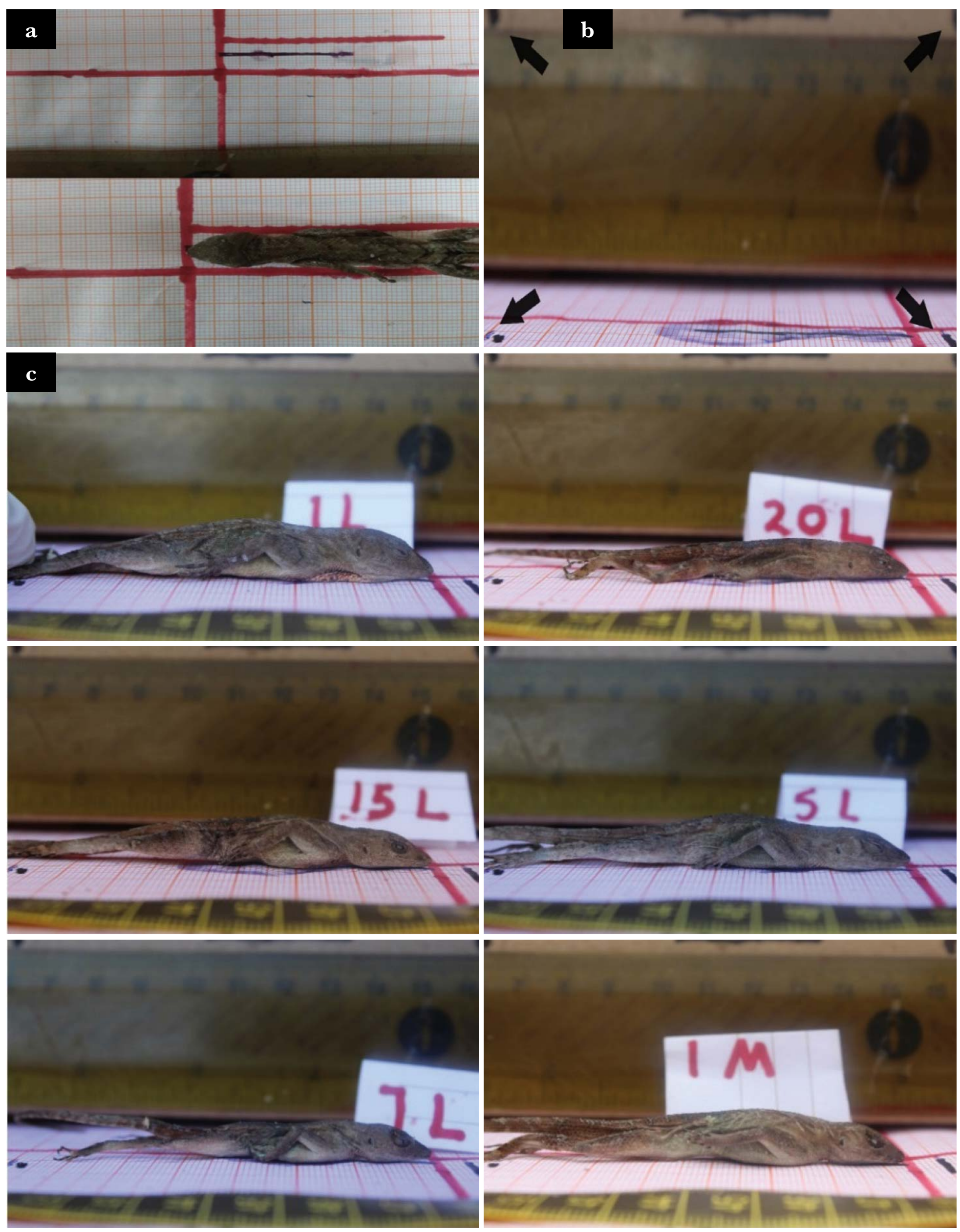

Figure 1. Stages of the photographic session of the *A. tropidonotus* specimens.

Landmark placement

All the photographs were digitized with the TpsDdig2 software (Rohlf, 2013). Landmarks were placed on the sides of the lizards' heads and semi-landmarks were set around the body outline (Vidal *et al.* 2006). Landmarks 7, 18 and 19 (red circles, Figure 2a) were placed at the intersection of the legs and the body; since they indicate the intersection of tissues, these are type-I landmarks. Eight type-II landmarks (white circles, Figure 2a) were positioned in local geometric structures: the maximum tip and the middle of the muzzle (landmarks 1, 2 and 13); the anterior and posterior ends of the eye and ear outline (landmarks 14, 15, 16 and 17); and the maximum point of the skull (landmark 10).

Lastly, semi-landmarks 3, 4, 5, 6, 7, 8, 9, 11 and 12 (yellow circles in Figure 2a) were placed around the outline of the animals' bodies, mainly in regions with no anatomical structures that could define type-I landmarks. Upon completion of landmark placement, the "Draw background curves" tool was used to connect the landmarks and estimate the consensus form of all samples, before starting the geometric morphometry analyses (Figure 2b).

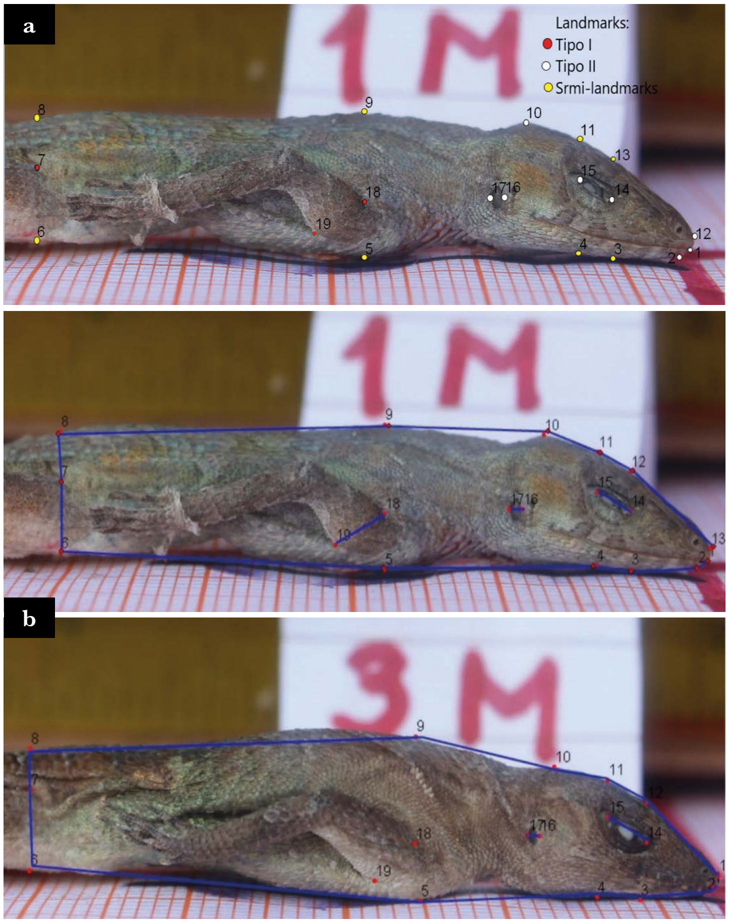

Figure 2. a) Placement of type-I and type-II landmarks and semi-landmarks in *A. tropidonotus* specimens; and b) estimation of the consensus form using the "Draw background curves" tool.

Geometric morphometry analyses

After the multivariate normal distribution of the data set was proven (comparing the expected theoretical distance distribution), the extreme or outlier values recorded were found to be below the expected value. Subsequently, the generalized procrustean analysis (GPA) was applied to create a "consensus" configuration representing the average of all the landmark configurations of the set of individuals. Morphometric analyses evaluate development pathways and structural design as a source of evolutionary diversification. Males and females were taken into consideration, since morphological variation, including sexual dimorphism (Benítez and Püschel 2014), is the focus of this study. Finally, the principal component analysis (PCA) was applied to explore form variation patterns between specimens. The MorphJ software was used to conduct these analyses (Klingenberg, 2011).

RESULTS AND DISCUSSION

Twenty-six specimens of *A. tropidonotus* were collected. Seven specimens (four ♂ and three ♀) inhabited tropical evergreen forests with a temperate climate (Fortín de las Flores ravine) and 19 (14 ♂ and five ♀) inhabited tropical deciduous forests with a tropical climate (Cerro de Lourdes).

The PCA produced 26 PC out of the 26 *A. tropidonotus* specimens, concentrating 34% of the variance. The lollipop graph showed that variation lies in landmarks 1 to 4 and 10 to 13, comprised of type-II landmarks and semi-landmarks (3-4 and 11-12), which are located on the head and muzzle. This set of landmarks and semi-landmarks showed variations in the shape of the head, the tympanic opening, and their relation to eye position (Figure 3). Out of the 26 deformation grids, the PC 2 landmarks expressed the full variance, particularly regarding the relationship between landmarks 1-4 and 10-13 (Figure 3).

The results obtained in this study showed intra- and inter-population variation resulting from sexual dimorphism. Two *A. tropidonotus* specimens of the same sex taken randomly from the same or different localities could have few morphological similarities. This variation could be due to environmental impact, low sample size, and sexual variation (Lafontaine *et al*., 2018), as well as to the gradual change of phenotypic traits associated with geographic distance and microenvironmental or clinal variation (Futuyma, 1998).

Structural microvariations were identified in the head and body outline and at the intersection of the leg and body tissues of both *A. tropidonotus* populations. These microvariations may be the result of the wide morphological variability and the distinctive phenotypic characteristics at the individual level, which are part of the life history that each individual has experienced in its microhabitat (Losos, 1994; Losos and Thorpe 2004). For example, larger *A. tropidonotus* specimens were caught in areas with disturbed vegetation where a larger presence and diversity of insects was observed. These feeding conditions may provide an advantage regarding food resources and show their potential morphological development. In contrast, the morphological development of some relatively small specimens caught in areas with a higher state of plant conservation and with a lower presence of insects could have been limited by atypical and unfavorable weather conditions (Losos, 2009).

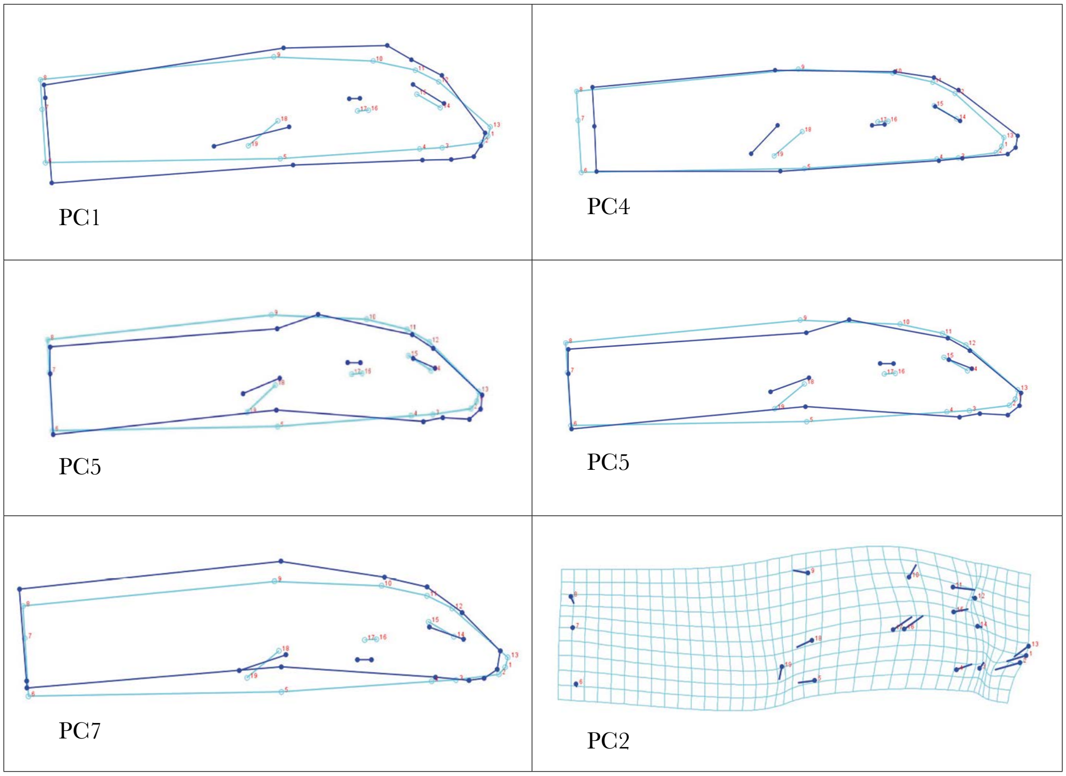

Figure 3. Lollipop graph comparing the principal components (PC) with shape variations in specimens of two populations of *A. tropidonotus* from contrasting environments, and deformation grid with PC2 showing the variation.

The study locations —in the central area of Veracruz, Mexico, where *A. tropidonotus* populations can be found— show variations in altitude, temperature, precipitation, humidity, soil types, vegetation, and food resources, which condition the differential development of *A. tropidonotus* individuals (Quatrini, 2001). This environmental variation in the geographic space inhabited by the *A. tropidonotus* populations impacts ecological processes and intra- and inter-population biogeographic patterns (Ghalambor *et al.*, 2006; Prates *et al.*, 2018). Therefore, the morphological variation observed may be associated with the selective and adaptive pressures resulting from the environmental, physical, and biological conditions experienced by each individual in its microhabitat (Hohenlohe *et al.*, 2010).

The intra-population variation of *A. tropidonotus* identified in this study may be the result of a population subdivision marked by physical barriers at the microhabitat scale (Campbell-Staton *et al.*, 2017). Climatic heterogeneity in their habitat, intra- and inter-specific relationships experienced by *A. tropidonotus* organisms, and geographic isolation of the populations shape the phenotypes of the species (Malhotra and Thorpe, 2000).

CONCLUSIONS

The intra- and inter-population morphological microvariations in five out of 19 landmarks evaluated in *A. tropidonotus* may be caused by the selective pressures that generate differences between individuals and sexes. Landmark 2, located at the edge and in the middle of the muzzle, was the trait that showed the greatest variation in both populations. Factors such as availability and quantity of prey may exert selective and adaptive pressure on this morphological trait of *A. tropidonotus* individuals —enough pressure to ensure that these sections of the muzzle are functional in each environment. Nevertheless, this hypothesis must be tested in future studies.

ACKNOWLEDGEMENTS

The authors would like to thank SEMARNAT for the Scientific Collection License no. SGPA/DGVS/001894/18 granted for the collection of the specimens used in this research.

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Colegio de Postgraduados

High-fiber diets for fattening pigs

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ABSTRACT

Objective: To establish the feasibility and benefits of implementing high-fiber diets for pigs.

Design/Methodology: A literature review of the practical application of pig diets with the inclusion of fibrous ingredients was carried out.

Results: The formulation of high-fiber diets for pigs maintains or improves productive performance and provides additional gut health benefits.

Study Limitations/Implications: Lack of information about the net energy and amino acid digestibility values of fibrous ingredients limits the proper formulation of pig diets.

Findings/Conclusions: The inclusion of high-fiber ingredients in pig diets can partially replace traditional ingredients, consequently reducing costs and providing health benefits.

Key words: forages, alternative ingredients, gut health.

Citation: Martínez-Aispuro, J. A., Figueroa-Velasco, J. L., Sánchez-Torres, M. T., Cordero-Mora, J. L., & Martínez-Aispuro, M.(2023). High-fiber diets for fattening pigs. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2419

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

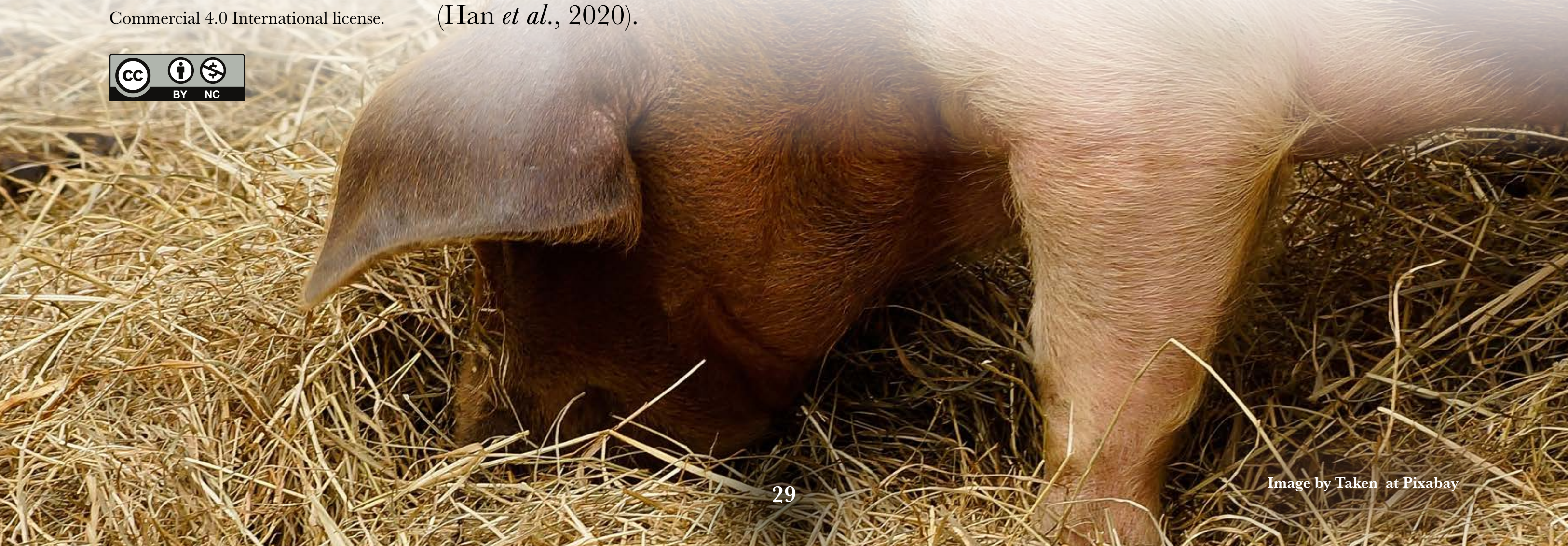
Received: October 31, 2022.
Accepted: June 19, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 29-35.

INTRODUCTION

The need to reduce pig feed costs and take advantage of special-priced ingredients has encouraged the use of high-fiber products and by-products from the agricultural industry (DDGs, hulls, bran, alfalfa, etc.). Overall, high-fiber ingredients are considered to have a low nutritional value in pig diets, given their lower energy and amino acid (AA) concentration in comparison with feeds with high starch or protein content and they have been attributed detrimental effects on the diet digestibility and productive performance (Agyekum and Nyachoti, 2017).

However, the use of dietary fiber in pig diets improves nutrient use and energy metabolism (Agyekum and Nyachoti, 2017), as well as the modulation of gut microbiota which leads to a reduction in antibiotic use, consequently improving productive performance and welfare (Han *et al.*, 2020).



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The effects of the source, type, and level of fiber inclusion on the gut health and productive performance of pigs must be identified (Agyekum and Nyachoti, 2017). Given the challenges involved in achieving predictable and profitable productive performance, the objective of this review was to establish the feasibility and benefits of implementing high-fiber pig diets.

Dietary fiber

Dietary fiber (DF) has attracted an increasing attention in pig nutrition. It is divided into soluble fiber (SF) and insoluble fiber (IF). Although it has traditionally been considered that DF can reduce nutrient digestibility and inhibit energy deposition, a higher amount of high-fiber feeds has begun to be used in pig diets (Li *et al.*, 2021), as a result of the potentially lower feed cost and DF nutritional assessment (Han *et al.*, 2020). The proper timing for the incorporation and inclusion of exogenous enzymes in the diet in order to reduce the anti-nutritional DF effects has also been assessed. However, the regulatory effect of DF in pigs is unclear; therefore, the wide range of fiber sources and diet composition must be taken into consideration (Li *et al.*, 2021).

In traditional studies about pig nutrition, DF was considered an antinutritional component, because it cannot be broken down by endogenous digestive enzymes and it can reduce nutrient digestibility (Jha and Berrocoso, 2015); these effects are associated with a detrimental effect on productive performance. Therefore, the DF ratio in commercial diets is minimal, resulting in the underuse and waste of high-DF ingredients.

Although pigs cannot digest DF, it can be fermented by gut microbes —mainly by bacteria in the large intestine. It can then be metabolized into volatile fatty acids (VFA: acetate, propionate, and butyrate), producing up to 30% of maintenance energy (Varel and Yen, 1997). VFA production and proportion varies according to the source and amount of fiber in the diet and the age/growth stage of the pig (Jha and Berrocoso, 2015).

Consequently, the sources and type of fiber must be identified and characterized, since soluble and insoluble components, viscosity, and other characteristics can modify the nutrients utilization and the development of the gastrointestinal tract (Hung *et al.*, 2020). Lyu *et al.* (2018) found a higher digestibility in diets with feeds whose SF concentration was higher than their IF. Feeds showing different SF/IF ratios could generate different productive responses. In addition, although the amount of IF leads to shorter digesta retention time, it may benefit the microbiota by reducing the adhesion of pathogenic bacteria to the mucosa (Molist *et al.*, 2014).

Overall, the risk posed by the inclusion of higher-DF content ingredients can be managed through formulations that take into consideration net energy and standardized ileal digestibility of amino acids (AA), exogenous enzymes addition, and feed processing.

Productive performance

High-fiber diets are commonly considered to cause a decrease in daily weight gain (DWG) and inhibit lean meat deposition in pigs (Wang *et al.*, 2016), mainly as a result of the decreased nutrient digestibility and energy deposition induced by the DF amount. Although Agyekum and Nyachoti (2017) mention that —whether the ingredients are rich in soluble or insoluble fiber or not— most studies show that DF does not impact productive performance (under certain assumptions).

The perceived impact of pig diets that include fibrous ingredients has changed and several authors point out that the said ingredients do not always have a negative response. Experiments conducted at the University of Alberta, Canada found that productive performance similar to that of pigs fed corn/soybean meal-based diets can be achieved with the inclusion of Distiller's Dried Grains with Solubles (DDGS) and canola meal (CM) in the diet of pigs at weaning (Landero *et al.*, 2013). In regards to finishing pig feeding, Jha *et al.* (2013) observed that similar productive performance of pigs fed lower fibrous ingredients can be obtained with the inclusion in the diet of up to 50% by-products (CM, flaxseed, and DDGS). It is important to note that in the abovementioned experiments, diets were formulated with an equal content of net energy and digestible AA. However, when Smit *et al.* (2014) assessed the CM inclusion (from 6% to 24%) in diets including 15% DDGS, they observed that increasing CM inclusion decreased gross energy digestibility, which could have reduced feed intake (FI), DWG, and feed efficiency.

Chen *et al.* (2013) attribute a decrease in nutrient digestibility as a result of the inclusion of alfalfa (one of the most commonly used forages in pig diets), mainly as a result of the presence of indigestible components. Chen *et al.* (2013) likewise assessed growing pigs whose diet included different levels of alfalfa (5%, 10%, and 20%) and wheat bran (5%) (13% NDF and 10% ADF; 15% NDF and 12% ADF and 19% DF and 14% ADF, respectively) and found that the inclusion of 5% alfalfa meal did not affect nutrient digestion and energy; meanwhile, acetate, propionate, and VFA concentration increased proportionally to the alfalfa concentration in the diet. Therefore, the authors inferred that alfalfa SF and IF play differential roles in nutrient digestion, which explains the variation in the intestinal flux of nutrients.

Chicory (*Cichorium intibus*) is a forage used in cattle production systems, with few reports about its use in pig feeding. The inclusion of chicory (4, 8, or 16%) in pig diets did not affect FI, DWG, or feed efficiency (Ivarsson *et al.*, 2011). The gradual inclusion (10 to 30%) of alfalfa and chicory in the diet, from the growing (40-60 kg) to finishing (80-100 kg) stages, obtained a similar DWG to that obtained with pigs fed grain-based diets. However, FI increased and feed efficiency decreased as the DF content increased (González *et al.*, 2020). It is worth mentioning that DF intake was similar in the chicory and alfalfa diet; nevertheless, the higher-SF intake in the alfalfa diet was associated with a negative decrease in productive parameters, since digestibility was higher in diets with ingredients whose concentration of SF was higher than IF (Lyu *et al.*, 2018). Likewise, Leivas (2017) reported that pigs fed alfalfa and chicory (20 and 30%) recorded a lower final weight; this difference was greater for alfalfa; although it did not have adverse effects on FI.

Wheat bran is one of the by-products commonly used in pig diets and it is characterized by its high fiber content. Therefore, it will be briefly addressed in this literature review, given the large amount of information available for this ingredient. Adding wheat bran to the feed of weaning pigs improves their gut health and modulates the activity and composition of their gut microbiota; however, its implementation in pig feeding shows that it is limited by its large amount of insoluble fiber, which is very resistant to natural degradation processes in the gut (Zhao *et al.*, 2018ab).

Sunflower meal is a fibrous by-product that can also be used as a good source of protein. On the one hand, Carellos *et al.* (2005) found that up to 16% sunflower meal can be included in the diet of finishing pigs without significant negative effects on productive performance. On the other hand, Cornescu *et al.* (2021) assessed different levels of crude fiber (3.5, 6.5, and 7.5%), adding alfalfa (4 and 6%) and sunflower meal (12 and 18%) to the diet of finishing pigs. Productive performance was not affected by either the source or content of the fiber; however, lean meat accumulation decreased.

High-fiber diets have also been used to reduce growth rate (and consequently energy density), in order to delay the market entry of pigs. Interestingly, research data show that the decrease in energy density in diets (from 3.32 to 2.86 Mcal of ME) did not result in a decrease in productive performance (Helm *et al.*, 2021a). Likewise, feeding finishing pigs with 12, 22, or 32% soybean hulls (SH) (15% NDF and 3.14 Mcal of ME; 20% NDF and 3 Mcal of ME; and 25% NDF and 2.86 Mcal of ME, respectively) caused a reduction in energy, without affecting productive parameters (Helm *et al.*, 2021a). Along with the reduction of energy, the inclusion of 20% SH (26% NDF and 2.99 Mcal of ME; Mauch *et al.*, 2018), 12 or 32% (13% NDF and 3.11 Mcal of ME; 23% NDF and 2.83 Mcal of ME, respectively; Helm *et al.*, 2021b) in the diets of finishing pigs limited the intake of diets with 20% and 32% SH, without negative effects on feed efficiency and DWG.

Carcass yield

High-NDF and ADF diets are generally associated with reductions in carcass yield (Mauch *et al.*, 2018). There is evidence that DF intake (10-30% chicory) increased digestive organ size and decreased carcass yield (Ivarsson *et al.*, 2011; Gonzalez *et al.*, 2020). Likewise, increasing the CM inclusion in the diet reduced carcass weight (Jha *et al.*, 2013).

When pigs are fed high-DF diets, the volume and weight of the viscera increase, resulting in higher energy and AA requirements for these organs. As a potential solution, high-DF diets that respect or increase the recommended net energy and digestible AA value could be formulated (Jha *et al.*, 2103). There is evidence that increasing DF does not impact carcass yield, as long as the AA concentration in the diet is not modified. Inclusion of up to 24% CM in diets including 15% DDGS affected neither carcass weight and yield, nor lean meat gain (Smit *et al.*, 2014). Likewise, adding up to 16% sunflower meal to the diet of finishing pigs did not modify carcass characteristics and performance (Carellos *et al.*, 2005).

Researchers agree that including more dietary fiber does not increase the thickness of subcutaneous fat, which is another important aspect to consider (Smit *et al.*, 2014; González *et al.*, 2020).

Gut health

One of the objectives of using high-DF in pig diets is to reduce the use of antibiotics and improve gut health (Helm *et al.*, 2021c). DF benefits the gastrointestinal system, improving the growth (DWG and feed efficiency) of pigs subjected to health challenges or stress

(Zhao *et al.*, 2018a). Including wheat bran in the diet of piglets positively modified their gut microbiota and butyrate production, consequently promoting gut health (Zhao *et al.*, 2018a). Likewise, piglets supplemented with lactoreplacer (0.2% pectin) had better health, DWG, and feed digestibility (Fleming *et al.*, 2020).

DF helps to decrease the incidence or severity of clinical pathological conditions, as a consequence of the relation of fiber with microbiota and the development of gastrointestinal diseases. Pigs fed diets containing low, medium (10% maize DDGS and 5% beet pulp) and high fermentation (10% beet pulp and 10% starch) fiber were subjected to a sanitary challenge with *Brachyspira hyodysenteria*. The percentage of pigs that developed clinical signs of dysentery was 85% (low fermentation fiber diet), 46% (medium fermentation diet), and 15% (high fermentation diet) (Helm *et al.*, 2021c). On the one hand, supplementing the diet of growing pigs with 0.5% inulin for 21 days increased cecal villi height and VFA production and decreased the apoptotic cell number and inflammatory cytokine secretion in the ileum and cecum. On the other hand, it also reduced the *Proteobacteria* concentration and increased the *Lactobacillus* concentration (He et al., 2021a). For their part, Wang *et al.* (2020) highlight that 2.5 g/kg of inulin reduced the *Echerichia coli* count in the colon, improving the functioning of the intestinal barrier (Wang *et al.*, 2020).

DF in pigs suppresses inflammatory response, improves morphology and intestinal barrier functions, and leads to beneficial changes in microbial fermentation (Wang *et al.*, 2020). According to Jarrett and Ashworth (2018), digesting fiber has beneficial effects on gut function and structure as a result of the VFA production. These changes include villus height and crypt depth (Liang *et al.*, 2014), as well as an increased antioxidant capacity of the intestine of pigs (Weber and Kerr, 2012). Hees *et al.* (2019) assessed the effect of supplying piglet diets with arabinoxylan and purified cellulose, observing that it led to an increase in the length and weight of the large intestine, as well as its VFA concentration.

Supplementation during gestation is another strategy to take advantage of the inclusion of DF, in order to improve gut health and, at the same time, obtain litter benefits. In contrast with a low-DF diet, providing a high-DF diet to pregnant sows led to a significant increase in the crypt depth of the ileum of newborn piglets. Similarly, the higher levels of acetate and isobutyrate that were found in the intestinal contents of the colon in piglets might be correlated with a better gut development (He *et al.*, 2021b). Finally, Li *et al.* (2020) point out that the incorporation of DF in the diet of pregnant sows improves the antioxidant capacity of piglets and reduces proinflammatory factors, which might be related to a positive modulation of the gut microbiota.

Adaptation

The progressive inclusion of DF enables a physical adaptation of the tract, through the enlargement of the stomach and the colon, but this adaptation period (AP) reduces carcass yield (Gonzalez *et al.*, 2020). The pig intestine needs a diet AP with different fiber types or levels, in order to gradually stabilize and increase DF digestibility (Zhao *et al.*, 2018b), since the AP duration affects the bacterial community and VFA profile in the pig caecum (Luo *et al.*, 2017). The AP to high-SF sugar beet was shorter than for pigs fed high-IF wheat bran, indicating that gut microbes adapted more readily to SF (Castillo *et al.*, 2007).

Growing pigs required more time to adapt to high-DF diets formulated with wheat bran, a phenomenon that is related to the gut microbiota activity (Zhao *et al.*, 2018ab), likely as a result of the 14-21-day period required to establish microecological homeostasis in the hindgut of pigs at weaning that were fed high concentrations of wheat bran (Castillo *et al.*, 2007).

CONCLUSIONS

General knowledge about the nutritional contribution and production benefits of including high levels of fiber in pig diets could help to predict production performance, meat quality, and health benefits, and to stop considering fiber as a negative aspect of diets, allowing the partial substitution of traditional ingredients with high-fiber feeds and consequently lowering their cost.

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Colegio de Postgraduados

Conjugated probiotics dispensed from birth to weaning for the survival of goat kids

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ABSTRACT

Objective: To prevent morbidities, mortalities and increase weight gain and growth of kids by administering oral probiotic conjugate (PC).

Design/Methodology/Approach: A randomized design comparing treatments (supplemented dose), percentage of morbidity and percentage of mortality. Goats were administered weekly from birth to 56 days of age. PC of *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were dosed weekly. Treatments, TC: Control, T2: 2.0 mL PC/kg body weight (BW). T3: 3 mL PC/Kg BW. T4: 4 mL PC/Kg BW.

Results: Diarrhea was present in: TC=16%, T2=1%, T3 and T4=0% (TC *vs.* T2, T3, T4, $P<0.05$). Mortality percentages: TC=17%, T2, T3 and T4=0% (TC *vs.* T2, T3, T4, $P<0.05$). T2 and T3 had an increase ($P<0.05$) of 1.9 kg weight gain (WG) *vs.* TC. T3 was the best treatment at 56 d ($P<0.05$).

Study Limitations/Implications: The doses used were defined based on other studies and experimental doses were used; the results consider that the facilities and management are optimal and in accordance with animal welfare standards.

Findings/Conclusions: The most adequate dose was 1014 CFU/kg BW since it improved WG and reduced mortality. It is suggested to compare oral doses of probiotics to reduce death due to enteric diseases.

Keywords: Goat kids, diarrhea, probiotic, mortality.

Citation: Hernández-Calva, L. M., Juárez-Mendieta, R., Villalobos-Peñalosa, P., Cortés-Roldán, P., Montalvo-Aguilar, X. G., Galaviz-Rodríguez, J. R. (2023). Conjugated probiotics dispensed from birth to weaning for the survival of goat kids. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2457

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: December 15, 2022.
Accepted: June 23, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 37-43.

INTRODUCTION

Intestinal probiotics adequately maintain the digestive health of animals and humans; its benefits depend on several factors, such as strains chosen, concentration, the viability of colony-forming units, and frequency of consumption (Salazar & Montoya, 2003). The excellent choice of a probiotic allows to maintain the health of the animals and improve the growth rate; there are reports were lactobacilli, and bifidobacteria have improved body weight and decreased mortality in piglets (Giraldo-Carmona *et al.*, 2015), lambs (Castro & Rodriguez, 2005) and kids (Markowiak-Kopeć & Ślizewska, 2019). The main reason for

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probiotic use is to avoid colonization of intestinal pathogens that adhere to the intestinal mucosa and to obtain a better immune response in the digestive tract (Delia *et al*., 2012). Antibiotics are commonly used to treat digestive disorders and control intestinal pathogens, although, its continuous use causes resistance to microorganisms and may end as residual compounds in food (Abd El-Tawab *et al*., 2016). In neonatal kids, the mortality rate is higher than 15% due to deficiency, respiratory and digestive diseases. The etiologies of the deficiencies are the lack of energy, protein, and selenium (Diaz-Sánchez *et al*., 2018). In the case of infectious diseases, protozoa, viruses, and bacteria participate (Salazar & Montoya, 2003). Probiotics in neonates, have generally been used to prevent digestive diseases and have been extensively studied in the calves, piglets, and lambs. However, the information published in kids is limited, some works describe the effect of probiotics in short times, but there are no studies that evaluate the use of probiotics in kids throughout the neonatal lactation cycle, which includes from birth to weaning. Therefore, the objective of the present study was to evaluate the oral use of a probiotic conjugate, administered weekly in kids from birth to 56 days of age.

MATERIAL AND METHODS

Study Site

The study was carried out at the "San José" Tepoxtla ranch, Yauquemehcan, Tlaxcala, Mexico. It is located between parallels 19° 21' and 19° 26' north latitude: meridians 98° 08' and 98° 13' west longitude. The altitude of 2,420 meters above sea level and temperature from 12 to 16 °C.

Herd Characteristics

The production unit has 300 French Alpine goats, with ages ranging from two to four years. The zootechnical purpose is the production of milk and kids for breeding and market supply. The animals are housed, and the essential rations are prepared based on the herd's physiological stages, formulated with oats, alfalfa, rootlets, barley, corn, and minerals. Initially, a total of 100 pregnant goats were selected, with breeding records, the females calved from December 2021 to February 2022. During deliveries, kids were identified, the weight and sex were recorded, and each animal was assigned to an experimental group.

Characteristics of the Probiotic Conjugate

The probiotic conjugate (PC) is a beverage; it was prepared in a cheese whey medium, including *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*. The probiotic conjugate consists of a certified guarantee of viable microorganisms of 106 colony forming units (CFU) per mL. The determination of the CFUs was performed with a colony counter (Kert-Lab. CM-1) described by Schell and Beermann (2014), in the Veterinary School of the Autonomous University of Tlaxcala's biochemical lab. During the kids' dosing, the probiotic complex was thawed and diluted in whey at 30 °C for 10 min. It was then cooled to room temperature and refrigerated during the oral administration process to the kids.

Experimental Distribution of Kids

The distribution of the kids at birth was done randomly in four treatments, considering the sex of the kid as a weighted variable, so that the groups would be homogeneous. The groups were distributed as follows:

TC: Control treatment, saline solution: 2.0 mL kg/body weight (BW), 12 goats and 12 kids.
T2: Dose of 2.0 mL PC/kg BW: 13 kids and 12 kids.
T3: 3.0 mL dose PC/Kg BW, 12 kids and 13 kids.
T4: 4.0 mL dose PC/Kg BW: 12 kids and 12 kids.

The first dosage of probiotics was given 24h after birth, and these were repeated every eight days for seven consecutive times, adjusting the dose of each treatment based on the weight of the registered kid. All the kids remained with their mothers, and the zootechnical management was similar in the four treatments. The zootechnical routine in the production unit is to disinfect the umbilical cord with a solution of iodine; it is ensured that the animal ingested colostrum during the first 24h of birth, the kids were identified with a plastic earring in the right ear, they were injected with two doses of selenium (25mg Se/kg BW) at three and 25 days after birth. The pens were cleaned daily, avoiding puddles or areas with humidity to elude the presence of coccidia. Milking of the goats was done two times a day, and a remnant of milk was left in the udder for the young's suckling. Additionally, there was a pen that allowed only the walk-through of the kids (Creep-feeding) to learn to ingest solid food that is high in protein and energy. Weaning was done at around 60 days of age.

The response variables measured during the 56 days of study were:

a) Presence of diarrhea: (number of kids with diarrhea/number of kids per treatment) $\times$100.
b) Percentage of total mortality: dead kids/weaned kids.
c) Findings in the necropsy.
d) the body weight gains (BWG).

This last variable was measured at days: 1, 8, 16, 24, 32, 40, 48, and 56 from birth.

Statistical Analysis

The presence of diarrhea and percentage of mortality were analyzed with the Kruskal-Wallis non-parametric test. Necropsy findings were interpreted visually. The BWG was analyzed with a 4$\times$8 factorial arrangement considering the four treatments and the eight weight sequences registered and identified as BWG.

Mean comparisons between treatments and measurements recorded over time were analyzed with the PROC MIXED SAS Statistical Analysis Program version 14.1 (SAS/STAT, 2015). Statistical significance was performed at the value of $P<0.05$ using the following statistical model:

$$Y_{ij} = \mu + T_i + M_j + TM_{ij} + \varepsilon_{ij}$$

Where: μ = mean; T_i = Effect of the *i*-th treatment (i = 1, 2,..., T); M_j = Effect of the *j*-th sampling time (j = 1, 2,..., M); TM_{ij} = Effect of the interaction of the *i*-th treatment for the umpteenth time; ε_{ij} = Random error for treatment *i* and time *j*.

RESULTS

Presence of Diarrhea and Mortality

The diarrhea presences were TC=16%, T2=1%, T3, and T4=0% (TC *vs*. T2, T3, T4, P<0.05).

Mortality percentages were TC=17%, T2, T3, and T4=0% (TC *vs*. T2, T3, T4, P<0.05).

All the diarrheas presented in the kids were semi-liquid, the perianal surface was dirty, with no foul odor, the excretion color was yellow-brown, indicative of colibacillosis. No diarrhea was melena type, ruling out the clinical presence of coccidiosis. The kids that presented the highest incidence of diarrhea were the TC, and all died. During the necropsy, clinical signs of dehydration, intestinal gasses, little presence of perirenal fat, and weight loss were observed.

Body Weight Gain

Table 1 shows the BWGs of the kids. There was no interaction effect between treatments and sampling times (P>0.05). Between the period times from 16 to 24 days, T2 and T3 increased (P<0.05) 1.9 kg in the BWG, unlike the TC. Then, during the period from 32 to 56 days, all the treatments supplemented with probiotics were better than the TC (~9.81 *vs*. 6.62 kg, P<0.05). Specifically, T3 was the best treatment, unlike T2 and T4, at 56 days (P<0.05).

Table 1. Body weight gains in kids Alpine-French supplemented with conjugate of probiotics*.

Supplement Days	TC 0 mL/kg BW	T2 2 mL/kg BW	T3 3 mL/kg BW	T4 4 mL/kg BW	SEM
1	3.02a	3.25a	3.67a	3.45ª	0.48
8	3.99a	4.98a	5.02a	4.47ª	0.48
16	5.40b	6.47a	6.87a	5.82ab	0.52
24	5.31c	7.42ab	8.28a	6.66bc	0.64
32	5.81c	8.21a	9.35b	7.58 a	0.48
40	6.25c	8.92ab	9.78a	8.01b	0.48
48	6.37c	10.21a	11.35b	9.16ª	0.52
56	8.07c	11.5a	13.07b	10.58ª	0.64

*Lactic acid bacteria *Bifidobacterium bifidum essensis*, *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with viable microorganisms of 10^6 colony forming units per mL. SEM=Standard error of the mean. Different letters between the same line show significant difference (P<0.05).

Table 2 shows the gains in body weight between males and females, regardless of the type of treatment, there were no significant differences by type of sex (P>0.05). It is worth mentioning that kids with signs of diarrhea, were not recorded their weight to affect the gains in body weight.

There was no mortality in the treatments supplemented with probiotics, while in the TC group was 12%, the causes of death are attributed to the onset of diarrhea, which in some way complicated the health of the animals with the presence of dehydration and body condition losses. The results indicate that the minimum dose of 1012 CFUs prevented colonization of pathogenic bacteria in kids. The objective of the study was to colonize the intestine with probiotic bacteria, prevent the invasion of pathogens, and prevent diarrhea from the first days after birth. Studies suggest the use of probiotics on the occurrence of diarrhea, decreased their presence in 37% (Görgülü *et al.*, 2003), recovering kids is usually after three days (Anandan *et al.*, 1999) as digestibility improves production and balance of the microbiota in the rumen; better BWGs were reflected in the kids (Galina *et al.*, 2009).

Other studies have administered single 2.3mL doses of CFU (*Lactobacillus* and *Lactococcus* spp.) per kg BW and no response was observed in the BWG (Gómez, 2020). Changes in microbial composition and population in the digestive tract led to a healthier condition. Probiotics are defined as dietary supplements containing potentially beneficial bacteria and yeasts and generally provide health benefits to the host. However, it also depends on the probiotic strain. The study by Santos *et al.* (2003) demonstrated that *L. acidophilus* CYC 10051, and *L. kefiranofaciens* CYC 10058 were more viable in intestinal health, although benefits were not always obtained in weight gains in kids (Anandan *et al.*, 1999; Ayişiği *et al.*, 2005; Görgülü *et al.*, 2003). Considering the digestive health benefits there are more advantages when using probiotics.

Other studies have evaluated food consumption, however, in this study, the consumption of milk and the sporadic ingestion of solid food was not measured, it is known that a good vitality of the kid helps it be more active to increase consumption. Baldwin *et al.* (2004) noted that kids fed only dairy diets, had limited rumen development and less capacity

Table 2. Body weight gains in three female and male Alpine-French kids without considering the assigned treatments

Number	Males*	Females*	SE
1	3.0833	3.4889	0.09
8	4.9167	4.7306	0.23
16	6.3083	6.2322	0.28
24	7.0833	7.1528	0.41
32	8.1667	7.9306	0.46
40	8.5833	8.4722	0.50
48	9.75	9.6111	0.39
56	11.00	11.3039	0.21

* Without significant statistical differences (P>0.05). SE=standard error.

than kids exposed to solid diets as a supplement. The efficacy of probiotic use can have coincidences or contradictions, Yoon, and Stern (1995) reviewed several studies with probiotics and concluded that at least 40% of the studies had positive responses to microbial supplements. Although, the answer depends on the environmental conditions (Donovan *et al.*, 2002, Krehbiel *et al.*, 2002) the comfort or overcrowding environment, immune status, hygiene conditions and the care given to the young (Cruywagen *et al.*, 1996). Concerning the differences between the sexes of the offspring; the study by Castillo-Rodríguez *et al.* (2013) reported higher weight in male goats than in neonatal females supplemented with probiotics, in the case of our study, there were no differences.

The continuous application of probiotics per week increased the management of the kids; perhaps it is assumed that there is a more significant economic expense. However, the result of not presenting any dead animal in the groups that were given the probiotics, justifies its continuous application and, it is considered an excellent strategy to maintain the survival of the kids. The effect is due to the continuous presence of probiotics in the digestive tract that causes dominance over pathogens and the dominance of probiotic conjugated microorganisms. Krehbiel *et al.* (2002) suggested that the efficacy of probiotics should be evaluated more in terms of their health benefits, as higher growth presented.

CONCLUSION

The seven-fold application from 10^{12} to 10^{16} CFU/kg BW is a conjugate of probiotics *Bifidobacterium bifidum Essensis*, *Lactococcus lactis*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*, improved health in kids and did not show any death. The dose of 10^{14} CFU/kg BW was the most suitable, improving the BWG up to 56 days. The dosage of these probiotics is suggested from the first day of birth to weaning of the kids, to reduce the causes of death from enteric diseases.

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Colegio de Postgraduados

Quality of a Cambisol at different times of agricultural use in the region of Los Ríos, Tabasco, Mexico

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ABSTRACT

Objective: To evaluate the quality of a Cambisol at different times of agricultural use in the Los Ríos region (RR), Tabasco, Mexico.

Design/Methodology/Approach: Four agricultural uses were selected on a Cambisol (CM): rainfed crop (RC), annual crop (AC), pasture (Pa), and secondary vegetation (SV). These were established at three different times (1984, 2000, and 2019) with four replications. Soil was collected using an auger at a depth of 0-30 cm. The physicochemical properties of the soil —such as texture (T), bulk density (BD), aggregate stability (AS), pH, electrical conductivity (EC), organic matter (OM), total nitrogen (N), phosphorus (P), and potassium (K)— were determined. A factorial analysis of variance was performed (significance level p=0.05). Variables that showed a statistically significant effect were subjected to Tukey's multiple comparison test (significance level p=0.05).

Results: Statistically significant differences ($p \leq 0.05$) were obtained for OM, P, K, and BD contents. The high OM content present in CM with SV in all years shows a better soil quality compared to CM with RC and AC. The high BD recorded in CM with Pa since 1984 shows soil quality degradation by compaction resulting from grazing due to extensive livestock farming.

Study limitations/Implications: Sustainable management practices are required to recover degraded CM.

Findings/Conclusions: OM and BD contents were the best quality indicators for the CM affected by the change in agricultural use and time of use in RR, Tabasco.

Keywords: Organic matter, Bulk density, Annual crops, Rainfed crops, Secondary vegetation.

Citation: Ramírez-García, A. R., Zavala-Cruz, J., Guerrero-Peña, A., Carrillo-Ávila, E., Sánchez-Hernández, R., Rincón-Ramírez, J. A., & García-López, E. (2023). Quality of a Cambisol at different times of agricultural use in the region of Los Ríos, Tabasco, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i6.2475

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: Jauary 19, 2023.
Accepted: June 29, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 45-52.

INTRODUCTION

Quality of soil is defined as soil's capacity to function and promote the sustainability of plants, animals, and human beings, as well as to maintain or improve the quality of water,

Image by PublicDomainPictures at Pixabay air, and the environment (Bünemann *et al.*, 2018). This functional concept allows us to assess this resource through qualitative and quantitative indicators (Pouladi *et al.*, 2020). These indicators integrate data on soil properties and characteristics in order to assess the response of soil productivity or functionality, and to indicate whether quality improves, diminishes, or remains the same (Schloter *et al.*, 2018).

Quality studies are necessary because soil is an essential resource in the creation of goods and services for ecosystems and for social wellbeing (Bünemann *et al.*, 2018), especially to meet water, energy, and food security needs (FAO and ITPS, 2015). However, soils have been subjected to degradation processes, mostly by erosion, and their health and crop yields have diminished as a consequence of the replacement of natural vegetation with agricultural uses (Jian *et al.*, 2020) and the implementation of unsustainable land uses and practices, which has led to infertility in one third of the world's soils (FAO and ITPS, 2015).

Soil degradation and the reduction in its quality threaten the future of human societies and the achievement of sustainable development objectives that guarantee the wellbeing of people and the environment (Keesstra *et al.*, 2016). Soils in 52.9% of the Mexican territory are degraded, and 70% of soils in the State of Tabasco show degradation related to unsustainable agricultural uses (Ortiz-Solorio *et al.*, 2011), with changes in physical, chemical, and quality properties (Geissen *et al.*, 2009). The objective of this work was to assess the physical and chemical quality of Cambisol with agricultural use at different times in the establishment of agricultural practices in the Los Ríos region, Tabasco, Mexico.

MATERIALS AND METHODS

Study area

The study was conducted in the municipalities of Balancán, Emiliano Zapata, and Tenosique in the Los Ríos region (RR), which is located east of the State of Tabasco in an area of 6234.2 km^2 (24.7% of the state). It borders the states of Campeche and Chiapas to the north and west, and the Republic of Guatemala to the east and south (Figure 1). Climates prevailing from north to south are warm sub-humid with summer rains (Aw2), warm humid with abundant summer rains (Am(f)), and warm humid with abundant rains all year round (Af(m)). Average annual rainfall varies between 1600 and 2000 mm and average annual temperature ranges from 26 to 28 °C (Aceves-Navarro and Rivera-Hernández, 2019). Cambisol (CM) covers 14% of the RR (Figure 1); it is found in alluvial plains and sandstone, shale, and limestone hills. This type of soil is used for cattle pastures, rainfed and annual crops, forestry plantations, oil palm, and secondary vegetation (Salgado-García *et al.*, 2017).

Selection of agricultural uses in Cambisols

Based on land use mapping created by Ramírez-García *et al.* (2022), In CM soils (Salgado-García *et al.*, 2017), four land uses were selected with three times of establishment (1984, 2000, and 2019): a) rainfed crops (RC) of corn, beans, squash, and watermelon; b) annual crop (AC) of sugarcane; c) pasture (Pa); and d) secondary vegetation (SV).

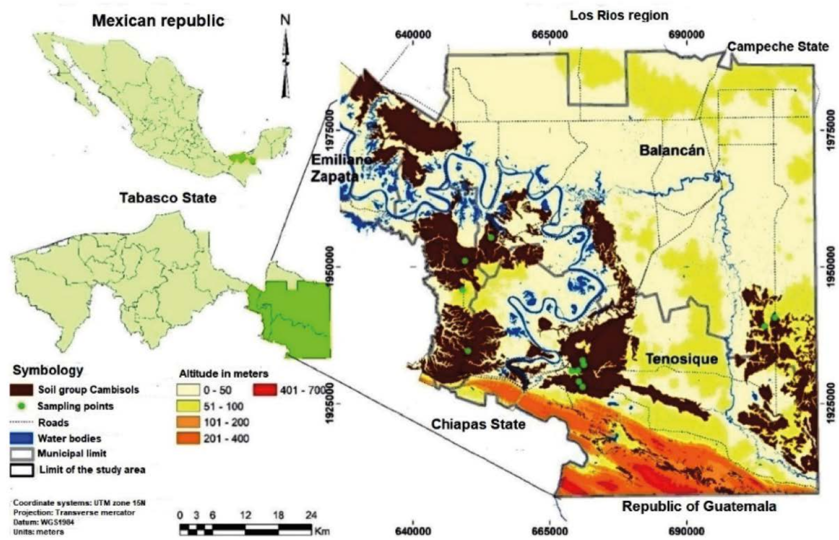

Figure 1. Location of the Los Ríos region, State of Tabasco, Mexico.

Soil sampling

For each land use —considering the three establishment times—, four sites were selected for sampling to estimate soil quality. At each site, a sample comprising 15 subsamples was obtained at a depth from 0 to 30 cm in an area of 400 m^2, following a zigzag pattern. The sample was then homogenized, and 1 kg of soil was set apart. To determine bulk density (BD), undisturbed soil was collected with a cylinder auger at 10 cm below the surface layer.

Laboratory analysis

Soil samples were air-dried at room temperature in the Laboratorio de Análisis de Suelos, Plantas y Aguas (LASPA) of the Tabasco Campus. For the physical and chemical analyses, samples were crushed, homogenized, and passed through a 2 mm sieve, or through a 0.5 mm sieve for the organic matter (OM) and total nitrogen (TN) analyses. To determine BD, soil samples were dried in an oven at 105 °C, then dry weights were calculated using the formula proposed by USDA (1999).

Aggregate stability (AS) was determined by the dry sieving method (Eynard, 2004). The following analyses were performed in compliance with the Official Mexican Standard NOM-021-RECNAT-2000: organic matter (OM) (Walkley and Black), total nitrogen (TN) (Semimicro Kjeldahl), phosphorus (P) (Olsen), potassium (K) (extraction with 1 N ammonium acetate, pH 7, and quantification by atomic absorption), texture (Bouyoucos method), pH (2:1), and electrical conductivity (EC) (1:5) (SEMARNAT, 2002).

Statistical analysis

A database of the soil physical and chemical properties used as quality indicators was generated, including four uses: RC, AC, Pa, and SV, with three times of establishment (1984, 2000, and 2019) and four repetitions. Land uses and establishment times were considered as factors (RC, AC, Pa, and SV), along with three levels (1984, 2000 and

2019). The factorial analysis of variance was carried out considering the CM physical and chemical properties as response variables with a significance level of p=0.05.

The assumptions of normality for the physical and chemical property values were verified with the Shapiro-Wilk test on residuals, and the Levene test for homoscedasticity was used on absolute residuals (Tassano *et al.*, 2021). Square-root, power, logarithmic, and Johnson transformations were applied using the Minitab Software v. 20.7 to achieve compliance with the assumptions on the variables (pH, EC, OM, N, P, K, BD, and AS) (Yañez-Vazquez *et al.*, 2018). A Tukey's multiple comparison test was conducted on variables having a significant effect on any of the factors or their interaction (Ajayi *et al.*, 2021). First, land uses were analyzed per year of establishment, then the same was done with all land uses in different years. Statistical analyses were processed using the statistical software InfoStat 2017.1.2. (Tassano *et al.*, 2021).

RESULTS AND DISCUSSION

Cambisol quality between agricultural uses with different years of use

The factorial analysis to determine the physical and chemical properties impacting CM quality with agricultural uses with different establishment times (years of use) showed statistically significant differences ($p \leq 0.05$) in OM content (Table 1). The highest OM content was observed in SV at all three times of establishment (1984, 2000, 2019) (Table 1). This is attributed to leaf litter and root residue decomposition by microorganisms, which favors an increased OM (Alabi *et al.*, 2019). In contrast, RC had very low OM values in 2019, due to continuous tillage practices that rapidly reduce OM in soil (Wang *et al.*, 2021). It should be noted that the CM of the RR showed medium to high levels of OM (2-6%) in its various land uses (SEMARNAT, 2002).

The highest K concentrations were found in RC of 2000, which can be attributed to the use of fertilizer (Alabi *et al.*, 2019), while Pa and SV of 1984 had the lowest values. With the studied land uses, CM showed low K values (<0.3 (cmol(+) kg^{-1}) (SEMARNAT, 2002).

Table 1. Mean values of the CM quality indicators between land uses with different establishment times (years of use) in the RR.

Year	Use	pH	EC (μS cm^{-1})	OM (%)	TN (%)	P (mg kg^{-1})	K (cmol(+) kg^{-1})	BD (g cm^3)	AE (WMD)	Clay (%)	Silt (%)	Sand (%)
1984	RC	6.8a	85.5a	2.5ab	0.1a	40.4a	0.3ab	1.2a	1.4a	36.1abc	31.8a	32.1ab
2000	RC	6.7a	89.6a	3.8ab	0.2a	35.0a	0.65a	1.1a	0.7a	62.3a	16.9a	20.8ab
2019	RC	6.1a	48.4a	1.7b	0.3a	37.8a	0.35ab	1.4a	0.8a	20.6bc	14.9a	64.4a
1984	AC	6.4a	71.0a	3.2ab	0.2a	30.4a	0.3ab	1.1a	0.8a	45.5abc	20.4a	34.1ab
2000	AC	7.0a	80.1a	2.5ab	0.2a	32.3a	0.33ab	1.3a	1.6a	51.3ab	21.4a	27.3ab
2019	AC	7.3a	107.1a	4.1ab	0.3a	27.3a	0.43ab	1.1a	1.4a	53.5ab	30.4a	16.1b
1984	Pa	6.1a	60.5a	2.9ab	0.2a	77.9a	0.18b	1.4a	0.5a	22.3abc	9.3a	68.4a
2000	Pa	7.1a	76.8a	3.9ab	0.3a	26.7a	0.23ab	1.4a	0.7a	27.3bc	26.8a	45.9ab
2019	Pa	7.1a	122.3a	5.3ab	0.3a	46.5a	0.38ab	1.1a	0.6a	52.1ab	19.4a	28.4ab
1984	SV	6.6a	86.4a	6.5a	0.3a	57.0a	0.2b	1.2a	0.9a	26.5abc	16.8a	56.7a
2000	SV	6.2a	60.2a	4.6ab	0.2a	60.2a	0.25ab	1.2a	1.1a	15.1c	10.8a	74.1a
2019	SV	6.5a	96.4a	5.6ab	0.3a	56.3a	0.35ab	1.0a	1.3a	58.6a	17.4a	24.0ab

In terms of grain-size percentages, sand —and to a lesser extent clay— showed statistically significant differences for the different uses and years. Changes in soil texture stem from the mineralogy of the parent material and the degree of weathering, because it is an inherent property of soil and is controlled by formation processes (Mulat *et al.*, 2021). This is confirmed by the varied-aged rocks where the CM of the RR develops, such as Holocene alluvium, Miocene detrital sediments, and Eocene limestones and marls (Salgado-García *et al.*, 2017).

The properties that did not show significant differences between the factors of use and establishment year were the following: a) pH, with a prevalence of neutral over moderately acidic (7.3 to 6.1) (SEMARNAT, 2002); acidic pHs in agricultural soils are due to intensive farming and the continuous use of acid-forming inorganic fertilizers (Mulat *et al.*, 2021); b) EC showed low values, characteristic of CM (Salgado-García *et al.*, 2017); this property helps crops assimilate other soil nutrients better (Navarro-García and Navarro-García, 2013); c) N presented low values —especially in RC and AC—, closely related to low to medium OM values, which reduces the mineralization level of N (Alabi *et al.*, 2019); d) P showed high values due to cattle excreta and applied fertilizers (Ramírez-Iglesias *et al.*, 2017); e) BD was higher in Pa, associated with compaction as a result of extensive livestock farming (Xu *et al.*, 2021); and f) AS had a low WMD in RC and Pa, which indicates a structural stability loss, while the increase in AC is related to the OM content; when attached to soil mineral particles, they form clay-metal-humic bonds that increase structural stability (De Freitas *et al.*, 2018).

Cambisol quality between agricultural uses

When analyzing the factor of land use type in the CM, significant statistical differences were observed in OM contents (Table 2): the highest was found in SV, and the lowest in RC and AC. This coincides with low records in cultivated soils, compared with derelict soils and soils with pastures (Alabi *et al.*, 2019; Wang *et al.*, 2021). Soil tillage increases susceptibility to erosion and OM loss (Jin *et al.*, 2021).

Cambisol Quality by agricultural use time

Data analysis of CM with land uses grouped by year (time of use) showed statistically significant differences in OM, P, K, and BD (Table 3). The highest OM contents were recorded for SV in 1984 and 2019, while the lowest corresponded to RC, AC, and Pa in 1984. These results evince the effect of use time on CM after 35 consecutive years with

Table 2. Mean values of CM quality indicators between land uses in the RR.

Use	pH	EC (μS cm^{-1})	OM (%)	TN (%)	P (mg kg^{-1})	K (cmol(+) kg^{-1})	BD (g cm^3)	AE (DMP)
RC	6.5a	74.5a	2.7b	0.2a	37.8a	0.43a	1.2a	1.0a
AC	6.9a	86.0a	3.3b	0.2a	30.0a	0.35a	1.2a	1.2a
Pa	6.7a	86.6a	4.0ab	0.3a	50.4a	0.26a	1.3a	0.6a
SV	6.4a	81.0a	5.5a	0.3a	57.9a	0.27a	1.2a	1.1a

Different letters in the columns indicate significant differences (Tukey, $p \leq 0.05$).

Table 3. Mean values of CM quality indicators for grouped land uses per year (use time) in the RR.

Year	Use	pH	EC (μS cm^{-1})	OM (%)	TN (%)	P (mg kg^{-1})	K (Cmol(+) kg^{-1})	BD (g cm^3)	AE (DMP)
1984	RC	6.8a	85.5a	2.5b	0.1a	40.4b	0.3a	1.2ab	1.4a
	AC	6.4a	71.0a	3.2b	0.2a	30.4b	0.3a	1.1b	0.8a
	Pa	6.1a	60.5a	2.9b	0.2a	77.9a	0.18a	1.4a	0.5a
	SV	6.6a	86.4a	6.5a	0.3a	57.0ab	0.2a	1.2ab	0.9a
2000	RC	6.7a	89.6a	3.8a	0.2a	35.0a	0.65a	1.1a	0.7a
	AC	7.0a	80.1a	2.5a	0.2a	32.3a	0.33b	1.3a	1.6a
	Pa	7.1a	76.8a	3.9a	0.3a	26.7a	0.23b	1.4a	0.7a
	SV	6.1a	60.2a	4.6a	0.2a	60.2a	0.25b	1.2a	1.1a
2019	RC	6.1a	48.4a	1.7b	0.3a	37.8a	0.35a	1.4a	0.8a
	AC	7.4a	107.1a	4.1a	0.3a	27.3a	0.43a	1.1a	1.4a
	Pa	7.0a	122.3a	5.3a	0.3a	46.5a	0.38a	1.1a	0.6a
	SV	6.5a	96.4a	5.6a	0.3a	56.3a	0.35a	1.0a	1.3a

Different letters in the columns indicate significant differences (Tukey, $p \leq 0.05$).

crops and pastures. The OM level decreased 55.8% compared to the CM with vegetation; on the contrary, the CM with SV denotes the soil conservation ecosystem service provided by natural vegetation, in contrast to cultivation systems (Jin *et al.*, 2021).

The highest P contents were observed in Pa in 1984, while the lowest were found in RC and AC of the same year (Table 3). The P value in Pa is consistent with reports on pasture soil by Burst *et al.* (2020) and can be attributed to the application of P mineral fertilizers (Alabi *et al.*, 2019) and to grazing residues (cattle manure and urine) that increase the availability of P in the soil (Ramírez-Iglesias *et al.*, 2017).

The K content was higher for RC in 2000, and lower for other uses in the same year (Table 3). The level of K in RC is associated with the frequent use of fertilizers (Pouladi *et al.*, 2020) that diminish when agricultural activity ceases (Xu *et al.*, 2021).

In terms of BD, the highest values for Pa were registered in 1984 (Table 3), which confirms the soil compaction attributed to cattle trampling for 35 years. This coincides with reports by Ajayi *et al.* (2021), who observed a BD increase from 1.4 to 1.6 g cm^3 in 24-year-old pastures. This process devalues soil quality, as it generates resistance to root growth; alters the distribution and connectivity of soil pores (Burst *et al.*, 2020); modifies water retention, infiltration, and availability, as well as soil aeration, affecting the edaphic biota (Wu *et al.*, 2021). In addition, compaction increases water erosion by surface runoff, as well as evaporation and soil temperature, and reduces its productivity (Burst *et al.*, 2020; Ajayi *et al.*, 2021). The low BD showed a statistically significant difference in AC in 1984 (Table 3); this was due to tillage carried out before planting sugarcane, since this activity decreases the BD (De Freitas *et al.*, 2018).

CONCLUSIONS

The high organic matter (OM) contents of Cambisols (CM) with secondary vegetation (SV) in the three assessed years (1984, 2000, and 2019) evidenced a higher soil quality compared to CM with rainfed (RC) and annual (AC) crops in the region of Los Ríos (RR), Tabasco. The high apparent density (BD) registered in CM with pastures (Pa) dating from 1984 indicate depreciation of soil quality due to compaction, as a consequence of extensive livestock grazing for 35 years. Fertilizer application is the cause of the high potassium (K) contents in RC of several years, as well as the high phosphorus (P) contents in Pa of 1984. The P levels in Pa are possibly supplemented by residues, such as cattle manure and urine. The OM and BD contents were the best quality indicators for the Cambisol soil due to the effects of agricultural use and time in the RR, Tabasco, since the highest and lowest values were obtained in 1984.

ACKNOWLEDGEMENTS

I am indebted to the Consejo de Ciencia y Tecnología (CONACyT) for the scholarship (CVU: 664717) granted during my training in the PhD program PRODOCAT, Campus Tabasco, COLPOS. We would like to thank the Tabasco Campus for the logistic support during our field and laboratory work, and the Laboratorio de Análisis de Suelos, Plantas y Aguas (LASPA) for the support during the analysis of soil quality.

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Colegio de Postgraduados

Adaptability of sugarcane (*Saccharum* spp.) clones, introduced by exchange to southeastern Mexico

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ABSTRACT

Objective: To evaluate sugarcane (*Saccharum* spp.) clones in the adaptability phase and select those with high field and factory yields that significantly exceed the commercial control clones.

Design/Methodology/Approach: Fourteen sugarcane clones were evaluated in a crop established for the first time and to which no cut has been made. The experimental design consisted of randomized complete blocks with four repetitions. The following agricultural characteristics were evaluated: stalk weight, population, and yield. The following industrial characteristics were likewise assessed: sucrose content, juice purity, and theoretical sugar production. Both values were compared with the values of the local clones MEX 69-290 and CP 72-2086 that were used as control.

Results: Statistical differences were found in agricultural and industrial characteristics between clones. In terms of stalk weight, the LTMEX 94-02 clone stood out, while, in population terms, the Mex 95-35 clone recorded the highest number of stalks per ha. Regarding field yield, the COLPOSCTMEX 09-1433 clone obtained significantly higher tonnage per hectare than the two control clones. Among the factory characteristics, the MEX 96-10 clone stood out with a significantly higher concentration of sucrose and with the highest juice purity. Finally, the COLPOSCTMEX 09-1433 clone had the highest theoretical sugar production value, which was statistically superior to the two control clones.

Study Limitations/Implications: The data were obtained from a crop established for the first time.

Findings/Conclusions: At least four clones showed high field and factory yields: COLPOSCTMEX 09-1433, LTMEX 94-02, MEX 95-59, and MOTZMEX 00-1192. All four showed a field performance that had better statistics than the control MEX 69-290; therefore, it would be appropriate to pursue its evaluation and multiplication during the semi-commercial test phase. However, since they were introduced, their evaluation should continue under the environmental conditions of the region.

Keywords: Selection, yield, sucrose, production, theoretical sugar.

Citation: Valdez-Balero, A., Juárez-López, J.F., Obrador-Olán, J.J., García-López , E., &Carrillo-Ávila, E. (2023). Adaptability of sugarcane (*Saccharum* spp.) clones, introduced by exchange to southeastern Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2480

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: January 24, 2023.
Accepted: June 25, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 53-61.

INTRODUCTION

The genetic improvement of sugarcane is carried out through the sowing of the hybrid seed, its subsequent evaluation, selection, and multiplication (at the beginning of the process), and the evaluation of the selected clones in 10 successive phases (Valdez *et*

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al.,1998). When the outstanding genetic material reaches advanced phases of evaluation, it is exchanged with other sugarcane regions of the country. It takes 10 to 13 years of continuous evaluation before a clone is finally recommended as a promising product (Valdez *et al.*, 1998).

The adaptability phase of sugarcane clones aims to determine the agricultural and industrial behavior of the selected materials in the experimental field (Flores, 2001). In the southeast of Mexico, sugarcane is grown in approximately 121,657 ha, 70% of whose surface is covered with three clones: Mex 69-290, CP 72-2086, and MEX 79-431 (MAM, 2022). The national average field yield is 67 t ha^{-1} (ATAM, 2021), while in the southeast of Mexico it is only 54.19 t ha^{-1} (COLPOS, 2021). Additionally, many sugarcane fields are old fields (with discontinued clones and low population densities), show disease problems, and have high percentages of clone mixing. This problem causes losses that harm producers and industrialists. In 2006, the Colegio de Postgraduados-Campus Tabasco began the Programa de Mejoramiento Genético for the improvement of sugarcane, with the objective of selecting clones with better field yields and sucrose content than currently cultivated clones. Sugarcane clones are the backbone of the supply area of sugar mills; however, they have an average useful life of 25 years, as a consequence of the appearance of new pests and diseases, among other causes (Martínez, 2017). The ideal sugarcane clone meets the needs of producers, field workers, and industrialists (Climaco and Ranjel, 1995). To solve the abovementioned problems, 14 sugarcane clones that have excelled in the selection process in other sugarcane regions of Mexico were evaluated during the adaptability phase.

MATERIALS AND METHODS

Experimental site location

The present work was carried out on a plot of the Unión Local de Productores de Caña de Azúcar C.N.C. of the Ingenio Benito Juárez, A. C., located in Poblado C-27, Cárdenas, Tabasco (18° 00’ 37” N and 93° 34’ 56” W).

Sugarcane clones evaluated

The clones were exchanged and selected in the Experimental Field of the Colegio de Postgraduados-Campus Tabasco in the Strain, Plot, and Furrow phase, according to the methodology recommended by this institution. The sugarcane clones to be evaluated in this research work are included in Table 1.

Table 1. Sugarcane clones introduced for exchange purposes and evaluated in the adaptability phase.

No.	Clone	No.	Clone	No.	Clone
1	MEX 95-03	6	EMEX 91-917	11	LTMEX 94-02
2	MEX 95-59	7	MEX 09-82	12	MEX 96-10
3	COLPOSCTMEX 09-1433	8	M 1658-78	13	MEX 69-290 (t)
4	EMEX 96-35	9	EMEX 91-117	14	CP 72-2086 (t)
5	MEX 95-35	10	MOTZMEX 00-1192		

Control clones 13 and 14 (MEX 69-290 and CP 72-2086) occupy the largest sugarcane cultivation area in southeastern Mexico.

Experimental design

A randomized complete block (RCB) experimental design with four repetitions was used, considering the clones as treatments, obtaining a total of 14 treatments: 12 clones from the selection process and two as control treatments. The experimental units consisted of twenty-four 12-m long furrows. The agricultural variables were evaluated in 4 central furrows of the useful plot, while the industrial variables were evaluated in the 12 lateral furrows.

Establishment and monitoring of the crop (experimental plot)

To guarantee the homogeneity between the clones, the material used came from the multiplication phase III in a 9-month-old crop established for the first time. The land preparation consisted of two fallowings, two harrowings, and one furrowing with 1.4 m between furrows and a depth of 40 cm. After planting and establishment, the technological package recommended to sugarcane producers from the southeastern region was applied.

Variables

The data of the variables were obtained when the crop established for the first time reached 15 months of age.

Agricultural variables

Population

The existing 2-m stalks from the central furrows of the experimental units were counted. The population of sugarcane clones were classified as very good (>80,000 stalks per hectare), good (79,000 to 60,000), regular (59,000 to 40,000), and poor (40,000) (IMPA, 1988).

Five repetitions of each of the abovementioned agricultural variables were taken in each of the experimental units and in each of the four blocks.

Stalk weight

The stalks of the four central furrows of each experimental unit in the four blocks were harvested and weighed. Less than 1 kg of stalk weight is considered light, 1 to 1.5 kg of stalk weight is considered medium, and more than 1.5 kg stalk weight is classified as heavy (IMPA, 1988).

Field yield

The averages of the stalk weight (tons of sugarcane per hectare) and population (number of stalks per meter) variables were used to estimate field yield. More than 130 t ha^{-1} is classified as an excellent yield clone, from 100 to 130 t ha^{-1} as a high-yield clone, 70 to 100 t ha^{-1} as a medium-yield clone, and less than 70 t ha^{-1} is classified as a low-yield clone (IMPA, 1988). The methodology recommended by IMPA (1988) was used to evaluate the agricultural variables.

Industrial variables

Samples were taken from the lateral furrows of each experimental unit and 20 stalks were sent to the laboratory of the Pdte. Benito Juárez, S. A. sugar mill. They were analyzed with the pol formula, from 11 to 15 months of age, and the following variables were determined in the stalk juice: sucrose content, purity, fiber content, reducing sugar content, and theoretical sugar yield. The last variable was calculated with the field yield and sucrose content values and was expressed in tonnes of sugar per hectare.

Statistical analysis

The information obtained for the evaluated response of both the agricultural and industrial variables was systematized and organized. The experimental information was subjected to an analysis of variance with the R ver. 4.1.1 statistical software for Windows.

RESULTS AND DISCUSSION

Stalk weight

There were statistical differences between the 14 clones evaluated regarding this variable (Figure 1). Clone 4 (EMEX 96-35) was the lightest and clone 11 (LTMEX 94-02) was the heaviest. Clones 11 (LTMEX 94-02), 3 (COLPOSCTMEX 09-1433), 2 (MEX 95-59), 10 (MOTZMEX 00-1192), and 9 (EMEX 91-117) were classified as heavy (>1.5 kg). The stalk weights of the first three were statistically equal to each other, with clone 11 being significantly heavier than clones 10 and 9 (Figure 1). The rest of the clones had statistically similar values and were classified as medium (1 to 1.5 kg) (IMPA, 1988) (Figure 1).

The stalk weight of sugarcane depends on the clone, the environmental conditions, and the follow-up given to the crop (IMPA, 1988).

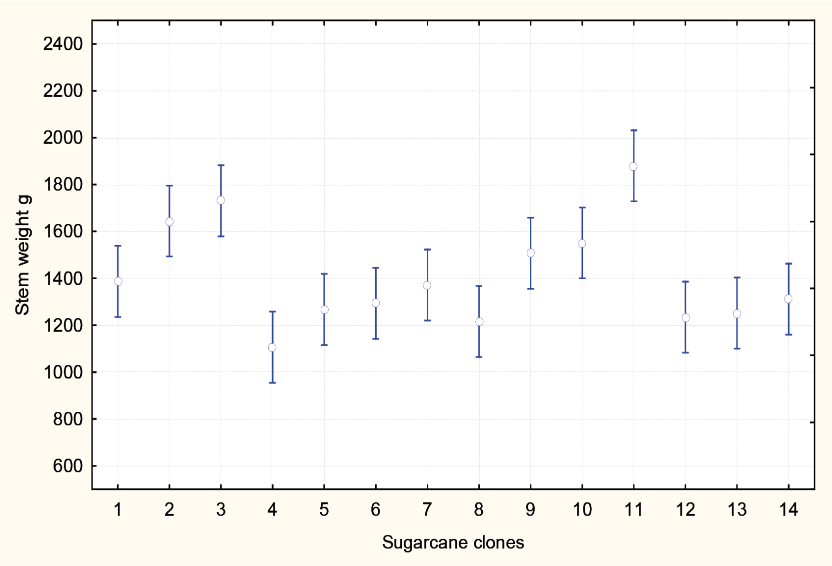

Figure 1. Stalk weight of 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

Population

Figure 2 shows statistical differences between the clones evaluated regarding the stalk population per hectare variable. Based on the IMPA measurement scale (1988), clone 5 (MEX 95-35) was classified as very good (>80,000 stalks per hectare), although its values were statistically equal to the other clones, with the exception of clone 12 (MEX 96-10), which recorded a significantly lower value. The rest of the clones fall within the good category (from 60,000 to 79,000 stalks per hectare) and clone 12 (MEX 96-10) belongs to the regular category (from 40,000 to 59,000 stalks per hectare).

Clones with more than 120,000 grinding stalks per hectare are not desirable. Overall, their thin and shorter stalks result in poor field yields.

Field yield

There were statistical differences between the 14 clones evaluated. Clones 3 (COLPOSCTMEX 09-14339), 11 (LTMEX 94-02), and 2 (MEX 95-59) had the highest yield, although their values were statistically equal to those observed in the 10 clones with the highest yield (Figure 3).

It is important to note that all the clones evaluated in this research work exceeded the average yield of the national mean (67 t ha^{-1}), as well as the average yield in southeastern Mexico (54.19 t ha^{-1}) (MAM, 2022). On the one hand, the field yield of clone 3 (COLPOSCTMEX 09-1433) statistically exceeded the yield of the two control clones (CP 72-2086 and MEX 69-290). On the other hand, five of the evaluated clones statistically exceeded the field yield of control clone 13 (MEX 69-290): clones 3 (COLPOSCTMEX 09-1433), 11 (LTMEX 94-02), 2 (MEX 95-59), 9 (EMEX 91-117), and 10 (MOTZMEX 00-1192), with yields of 136,225, 130,850, 126,300, 119,225, and 114,775 t ha^{-1}, respectively.

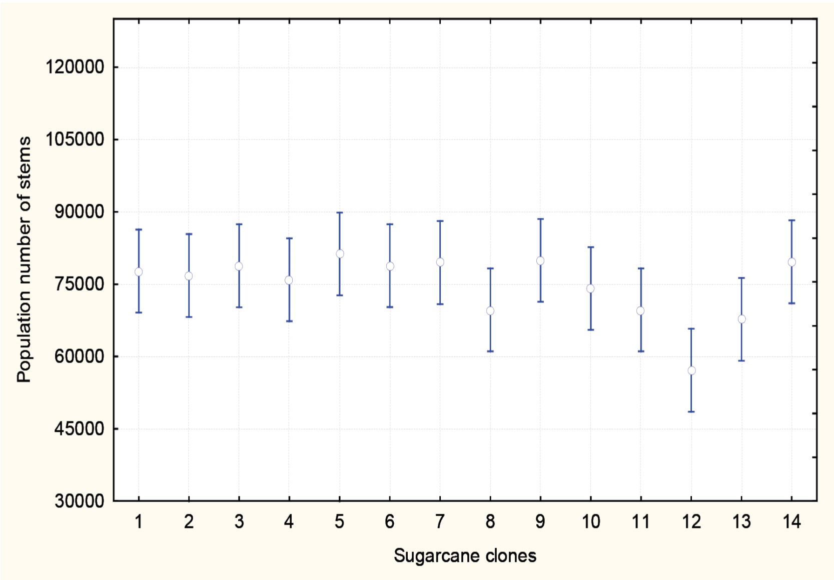

Figure 2. Stalks population per hectare in 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

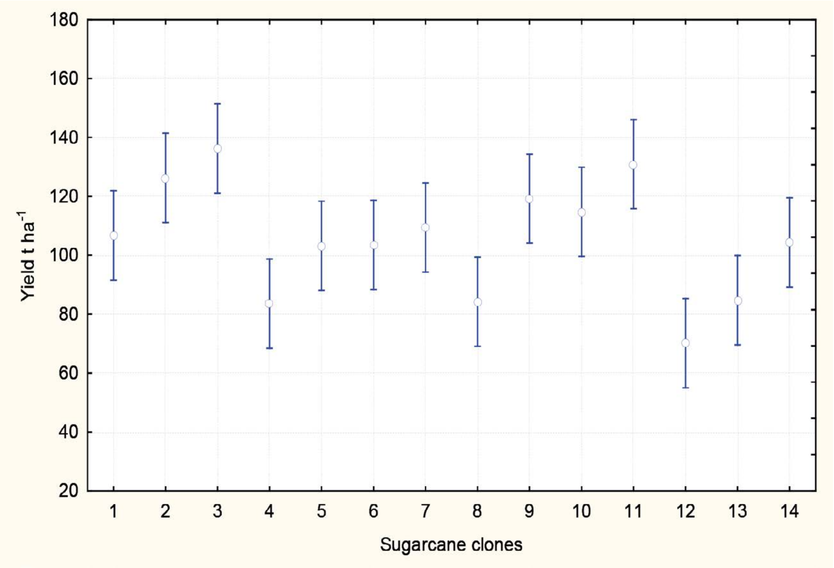

Figure 3. Yield in t ha^{-1} of the 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

Yield is a compound variable in which substantial changes can occur from one cycle to another (first cycle to second regrowth), mainly influenced by the environment, the genetic characteristics of the clone, and crop management. In sugarcane fields, yield is expressed in tons of sugarcane per hectare (García, 1981).

Industrial parameters

Sucrose

There were statistical differences regarding the sucrose percentage between the 14 clones evaluated (Figure 4). Clone 12 recorded the highest percentage of sucrose concentration (MEX 96-10), which was significantly higher than the others. Clones 2 (MEX 95-59), 6 (EMEX 91-917), 7 (Mex 09-82), and 9 (EMEX 91-117) showed the lowest concentration, with values which were statistically equal between each other and significantly lower than those found in the other clones. Both clones 12 (MEX 96-10) and 3 (COLPOSCTMEX 09-1433) recorded higher sucrose contents than the two control clones.

Sugarcane is considered mature or adequate for industrial processing, as long as its juice has a ≥13% concentration of sucrose (Larrahondo and Villegas, 1995). Ripening is the process by means of which sucrose accumulates in the stalk and it requires a decrease in the speed of growth to favor the accumulation of the sugars produced during the photosynthetic activity (Larrahondo, 1995). Martínez (2012) pointed out that the harvest of the clone must take into account its maturity, in order to take advantage of the maximum concentration of sucrose in the stalk and transform it into a greater amount of sugar. García (1981) mentions that sucrose is the sugar in sugarcane juice and other vegetables: it is a disaccharide (chemical formula: $C_{12}H_{22}O_{11}$) produced by condensation of equimolecular amounts of glucose and fructose. Clones with low levels of sucrose have high contents of reducing sugars (Larrahondo, 1995).

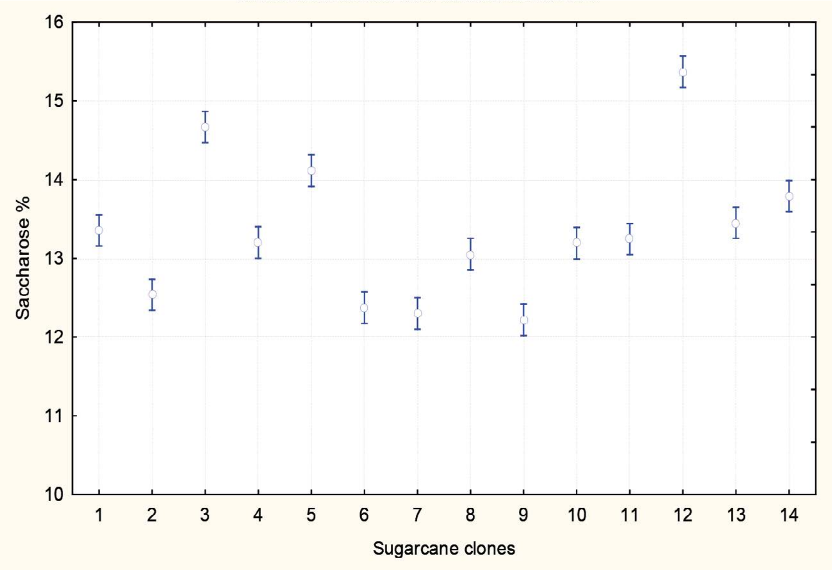

Figure 4. Sucrose concentration of 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

Juice purity

Statistical differences were recorded in juice purity between the clones evaluated. Clone 12 obtained the highest results (MEX 96-10), with a statistically similar value to clone 3 (COLPOSCTMEX 09-1433); both had significantly higher values than the rest (Figure 5). Clones 7 (MEX 09-82), 9 (EMEX 91-117), and 6 (EMEX 91-917) showed significantly lower juice purity.

García (1981) indicated that juice purity is the percentage of solids dissolved in the sugarcane juice. For his part, Larrahondo (1995) defines it as the percentage ratio between

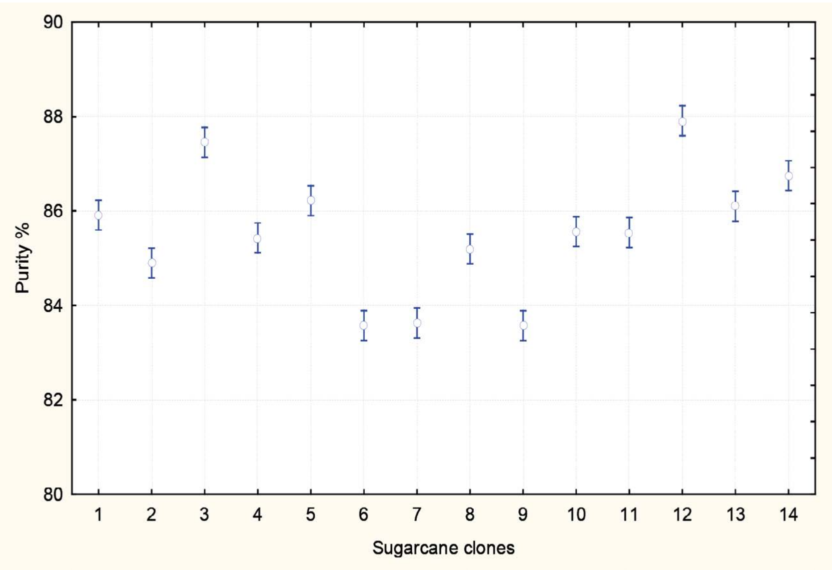

Figure 5. Juice purity in 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

the sucrose in the juice and the °Brix. The selection and genetic improvement program must discard individuals whose juice purity is below 85%.

Theoretical sugar production

Statistical differences were found among the 14 clones when the theoretical sugar production was determined (Figure 6). Clone 3 (COLPOSCTMEX 09-1433) showed a significantly higher theoretical sugar content (19.99 t ha-1), which was statistically different from the others, except for clones 2 (MEX 95-59) and 11 (LTMEX 94-02) (Figure 6). Clone 12 (MEX 96-10) showed the lowest production (10.77 t ha^{-1}); however, it had a statistically similar value to the other clones (1, 4, 5, 6, 7, 8, 9, 13, and 14) (Figure 6).

García (1981) indicates that theoretical sugar production is the amount of sugar that the factory can recover from a clone, based on the specific weight of the sugarcane stalks, as well as the extracted juice, whose sucrose concentration is known. Larrahondo (1995) mentions that the quality of a clone is determined at the time of milling, based on the amount of recoverable sugar obtained per tonne of milled sugarcane. The theoretical sugar production is made up of two parameters: the agricultural (yield) and the industrial (sucrose) parameters. As the basis on which sugarcane producers paid, it is extremely important for the recommendation of a clone for commercial cultivation. Clones 3 (COLPOSCTMEX 09-1433) and 11 (LTMEX 94-02) showed a statistically higher theoretical sugar yield than control clone 13 (MEX 69-290). The theoretical sugar concentration in clone 3 was significantly higher than in the two controls (MEX 69-290 and CP 72-2086). However, the values found for all clones are within the acceptable range (IMPA, 1988).

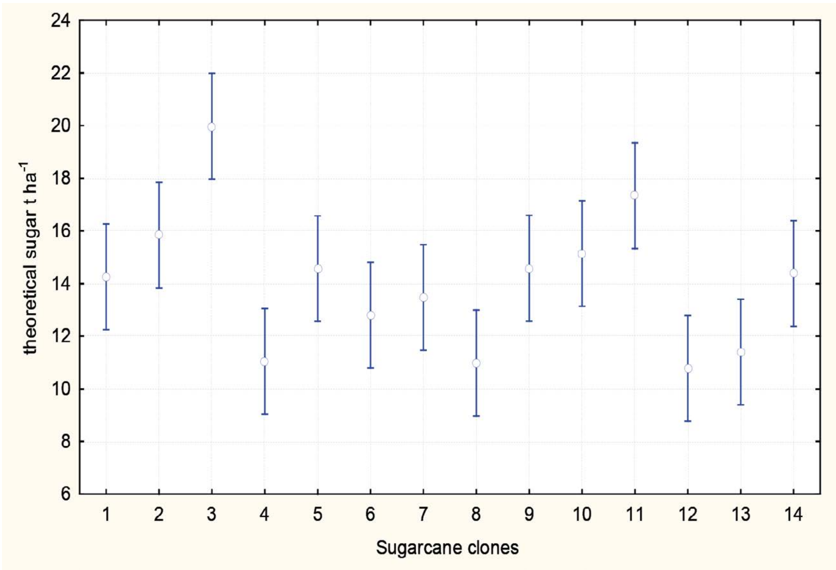

Figure 6. Theoretical sugar production of 14 clones evaluated in the adaptability phase (vertical bars indicate the 95% confidence interval).

CONCLUSIONS

Four of the sugarcane clones evaluated in this research work showed high field and factory yields: COLPOSCTMEX 09-1433, LTMEX 94-02, MEX 95-59, and MOTZMEX 00-1192. The COLPOSCTMEX 09-1433 clone significantly outperformed the two control clones (MEX 69-290 and CP 72-2086) in theoretical sugar production. The LTMEX 94-02 clone significantly outperformed the control clone MEX 69-290 in the said variable. In terms of field yield, the clone COLPOSCTMEX 09-1433 significantly outperformed the two control clones. All four clones produced statistically higher values than those observed in the MEX 69-290 clone. Consequently, the evaluation and multiplication of the four clones must be continued during the semi-commercial test phase, along with their observation in the field. However, since they were introduced to the area, their ongoing evaluation should continue under the usual environmental conditions of the region.

ACKNOWLEDGEMENTS

The authors would like to thank to Unión Local de Productores de Caña de Azúcar C.N.C. of the Pte. Benito Juárez, A. C. sugar mill who financed the "Generación de nuevos clones de caña de azúcar" project.

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Socioeconomic characteristics of the broad bean (*Vicia faba* L.) (Fabaceae) production in the northeastern region of the State of Puebla, Mexico

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ABSTRACT

Objective: To determine the economic importance of broad bean (*Vicia faba* L.) (Fabaceae) cultivation in four municipalities of the northeastern region of the State of Puebla and to determine the knowledge level of growers regarding the symptoms, control methods, and damage caused by the chocolate spot.

Design/Methodology/Approach: Semi-structured interviews were applied focusing on production systems, phytosanitary management, and acceptance of biopesticides. The resulting information was subjected to a database descriptive analysis using the Microsoft® Excel package.

Results: The data output shows the importance and extension of the sowing, along with the phytosanitary problems faced by regional broad bean growers and finally the acceptance of biopesticide as a method to control the chocolate spot.

Study Limitations/Implications: There is not enough information and documents regarding the importance of the regional cultivation of broad bean as a source of sustenance.

Findings/Conclusions: The economic importance of broad bean cultivation was determined, along with the damages caused by the chocolate spot infection and the production systems used by the regional grower families.

Keywords: Production systems, broad bean, biopesticides, and chocolate spot.

Citation: Mora-Baez, G., Torres-Rueda, L., Morgado-González, A. Harris-Valle, C., & Nava-Diaz, C. (2023). Socioeconomic characteristics of the broad bean (*Vicia faba* L.) production in the northeastern region of the State of Puebla, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2493

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: January 27, 2023.
Accepted: May 29, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 63-68.

INTRODUCTION

Broad beans (*Vicia faba* L.) (Fabaceae) is a species with worldwide importance as food (Rojas-Tiempo *et al.*, 2012). It is the fifth most important crop in Mexico and it is grown in the states of México, Puebla, Tlaxcala, Veracruz, and Hidalgo. However, the most important Mexican state regarding broad been production is Puebla with 16,083 ha, most

Image by Alicia Cortés at Pixabay

of which belong to several municipalities in the northeastern area of that state. Domestic yield ranges from 1.3 to 6.6 t·ha^{-1} (SIAP, 2019). Yield loss is associated with the presence of pests (Lake *et al.*, 2019), particularly the *Botrytis fabae* Sardiña fungus, commonly known as chocolate spot. It causes necrotic spots in stems, leaves, flowers and pods and it can reduce annual production by up to 60% (Gahukar, 2012). The main strategy employed to control this pathogen is the use of highly toxic fungicides; however, pest management can increase production costs (Espinal *et al.*, 2010). Therefore, farmer lore regarding the management of broad bean crops should be documented, particularly, the way the control the chocolate spot, since their experience is a source of information for the organization and operation of traditional agricultural production systems in the northeastern region of the State of Puebla. Specifically, the study focused on the municipalities of Libres, Zaragoza, Tlatlauquitepec, and Zacapoaxtla, whose production makes Puebla one of Mexico's main broad bean growers.

MATERIALS AND METHODS

A semi-structured interview was designed to collect information from n=20 randomly selected growers from the municipalities of Zaragoza, Zacapoaxtla, Libres, and Tlatlauquitepec, in the State of Puebla. In total, n=80 growers were interviewed. The survey covered the characteristics of agricultural production systems, including years that the crop has been grown in the plot, obtaining, choosing, and storing seeds, established surface, use or management of fertilization programs, economic aspects (investment on the establishment and handling per hectare), yield, and markets in which the grain or pods are commercialized. Likewise, the answers to some questions provided the research team an overview of the identification of the pathogen (chocolate spot), its phytosanitary control, resulting loses, and finally the interviewees' opinion about the acceptance or implementation of biopesticides.

The municipalities were chosen according to their production record in the state's northeastern region. The surveys were carried out on May 2021. The resulting information was used to develop a database, which was subjected to a descriptive analysis (through the development of bar graphs and tables), using Microsoft® Excel.

RESULTS AND DISCUSSION

Puebla is one of the main growers of broad beans and most of the interviewees declared that they have grown this species for more than ten years (Rojas-Tiempo *et al.*, 2012) (Figure 1).

The interviewees from the municipalities of Zaragoza and Zacapoaxtla grow broad beans for self-consumption, while in Zacapoaxtla, Zaragoza, and Tlatlauquitepec, growers sell their production in the local area. Growers from Tlatlauquitepec, Zaragoza, and Libres sell their product on the regional market, while 97% of the production from Libres is sold in the national market.

Broad beans are the seventh most important crop in the world (Pérez, 2014). They have played a major role in the crop pattern of the study region for several years. Their sales in various markets provides an income for the local families, particularly in the

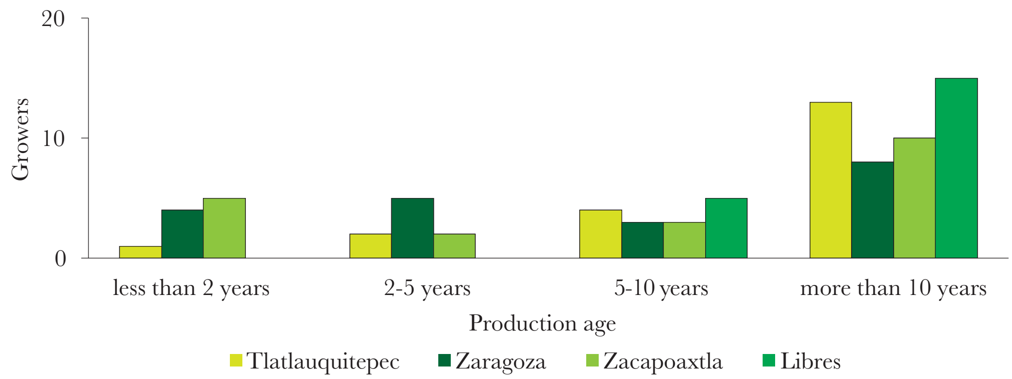

Figure 1. Years that growers from four municipalities in northeastern Puebla have cultivated broad beans.

municipality of Libres, where 95% of the growers mentioned it as their main source of income. Nevertheless, this agricultural activity is not the main source of income for 85, 80, and 70% of the growers from Tlatlauquitepece, Zaragoza, and Zacapoxtla, respectively, given its low yield and the presence of diseases, which matches the findings of Doussoulin *et al.* (2015).

To establish the crop, 85 to 100% of the growers use native seeds and only 5% (from Zaragoza and Zacapoaxtla) acquire commercial seeds. This situation proves that growers still select and preserve local germplasm, based on traditional lore, which they have perfected through empirical experimentation. This practice contributes to the in situ preservation of this species (Díaz-Bautista *et al.*, 2008). This study found out that 93% of the farmers believe that seed health is the main criterion for the selection of the biological material that will be used in the next production cycle, while 55% consider that both the health and the size of the seeds are important. Additionally, Pérez-López *et al.* (2015) suggest that the selection process should take into consideration the phenotypical expression (stem length, branches per plant, pods per plant, seeds per pod, and weight of 100 seeds).

Since they sell all their harvest, 65, 85, and 80% of the growers surveyed in Tlatlauquitepec, Zaragoza, and Zacapoaxtla, respectively, buy seeds from other growers to estalish their crops.

In the case of the municipality of Libres, 100% of the growers mentioned that they sow seeds from the previous harvest, which provides an opportunity to keep and safeguard local resources, in contrast with modern agriculture which has reduced regional genetic diversity (Duc *et al.*, 2010). Rojas-Tiempo *et al.* (2012) mention that the communities that make up the study area use two types of broad beans, identified by the growers as tarragona and cochinera (according to their size). Based on the classification proposed by Cuberto (1974), they belong to the minor and quina varieties, respectively. In a personal communication, growers mentioned that they use tarragona seeds. In this sense, the northeastern region of Puebla is characterized by the use of local cultivarls, such as tarragona, parraleña, and cochinera (Herrera, Alvarado, Cabrera, Hernández, and Guevara, 2020).

In Tlatlauquitepec and Zaragoza, each growers sows from 1 to 2 ha, while most growers from Zacapoaxtla grow broad beans in less than 1.0 ha. This information is related with the exclusive use of the production for self-consumption, as well as with the technification level, likely because their plots have scarce agronomical management. In the municipality of Libres, 80% of the growers cultivate broad beans in 2 to 4 ha. Although the only growers that establish more than 4 ha are located in Zaragoza and Libres, the highest yields per hectare were recorded in Libres (200-300 kg), followed by Tlatlauquitepec (150-250 kg). Growers from Zaragoza obtain a 100 to 200 kg yield, while the lowest yield is reported in the municipality of Zaragoza (Figure 2). The latter yield is lower than the 300 kg reported by Rojas *et al.* (2012) in the evaluation of a broad bean production system in which fertilization technological packages were implemented.

The economic investment per hectares varies between municipalities. In Zaragoza, Zacapoaxtla, and Tlatlauquitepec, investment is below MXN$2,500 ha^{-1}, while it exceeds MXN$4,000 ha^{-1} in the municipality of Libres. Growers from Tlatlauquitepec and Libres use products to disinfect broad bean seeds, which is not the case in Zaragoza and Zacapoaxtla; the consequent presence of phytopathogens might explain the yield reduction (Orozco and Zúñiga, 2020).

Chocolate spot is considered one of the main broad bean diseases, both in Mexico and the rest of the world (Torres *et al.*, 2006). Ninety-one percent of the growers can identify the disease's typical symptoms; therefore, 75 to 85% of the interviewees from the municipalities of Tlatlauquitepec, Zacapoaxtla and Libres carry out preventive treatments, while 75% of Zaragoza's growers do not control the pathogen in any way. The economical resources that are allocated to the control are very low (MXN$325.00 in Zacapoaxtla, MXN$375.00 in Zaragoza, MXN$650.00 in Tlatlauquitepec, and MXN$800.00 en Libres). Perhaps this is one of the reasons behind the 50% or higher loses of economic yield, which matches the findings of Espinal *et al.* (2010), who mention that *B. fabae* reduces yield by up to 60%.

In this context, 100% of the growers in Tlatlauquitepec, Zaragoza, and Zacapoaxtla mentioned that they are unaware of any specific pesticide that controls chocolate spots, while 15% of the growers form Libres mentioned at least one. Meanwhile, 90 and 95%

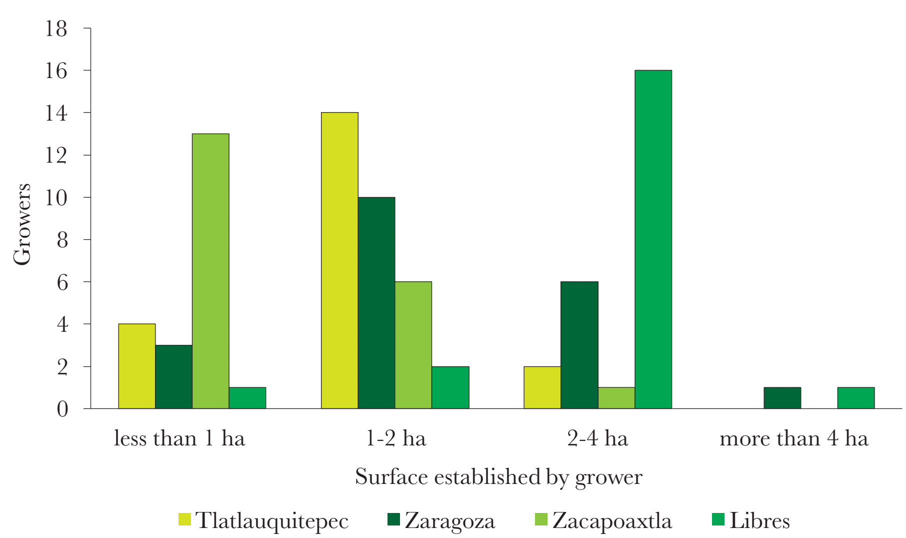

Figure 2. Area established with broad bean per grower in four municipalities of the northeastern region of Puebla, Mexico.

of the growers from Tlatlauquitepec and Libres, respectively, declared their interest and willingness to use some kind of biopesticide, as long as it reduces production costs and maximizes their yield. For their part, 75 and 8% of the growers of Zacapoaxtla and Zaragoza showed no interest in biopesticides. Nevertheless, Espinal *et al.* (2010) have reported that the application of *Trichoderma inhamatum*-based products to broad bean plants with chocolate spots symptoms improves the weight and leght of the seedlings, the numer and weight of the pods, the number of grains, and the fresh and dry weight of the grains, proving that agroecological products can efficiently control pathogens.

Likely due to the lack of technical consultancy services, some growers declare that they are not interested in alternatives for the control of chocolate spot; however, those who did show interest might act as models for the incorporation of biopesticides and positive regional results could convince their colleagues to adopt these alternative control methods. According to Nava-Pérez *et al.* (2012), the development of new biopesticides stimulates agricultural modernization. In agricultural production, biopesticides are ideal substitutes for traditional and highly toxic chemical products (Leng, Zhanhg, Pan, and Zhao, 2011).

CONCLUSIONS

Broad bean cultivation is not a recent practice in the study region: it has been part of the cultivaton pattern for over ten years and the seeds that are sown are obtained from the previous harvest in the same locality. As a consequence of its low yield. this agricultural activity is not the main source of income for the growers of some municipalities.

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Acarapis woodi (Rennie), honey bee endoparasite: from the pest of the century to a forgotten case in Mexico

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ABSTRACT
Objective: To determine the incidence of *Acarapis woodi* (Rennie) infestation in the tracheae of honey bees (*Apis mellifera* L.) from colonies not previously treated against the *Varroa destructor* Anderson and Trueman mite and to discuss the current status of *A. woodi* in Mexico, based on our observations and historical information.
Design/Methodology/Approach: Samples of the thoracic tracheae of honey bees from colonies never treated against the *V. destructor* mite were taken in search of *A. woodi*. A 20-worker sample was collected from each colony, preserved in 70% ethanol, and examined no later than two days after the collection. Foraging workers were selected from the lateral combs, the entrance of the hive, and the inner lid, the places where the oldest bees can be found and which have a higher infestation probability trend. The specimens were diagnosed using the dissection technique and observed under a microscope.
Results: None of the examined honey bees' tracheae —and therefore none of the colonies— were infested.
Study limitations/Implications: Although only one apiary was studied, a general trend can be observed in Mexico and many other countries in the Americas, especially in tropical and subtropical sites: after a shock wave of *A. woodi* infestations, they were less frequent, until their detection became very difficult.
Findings/Conclusions: The decreasing prevalence of *A. woodi* has been attributed to climatic preferences and treatments against *V. destructor*. However, this reduction could be the result of resistance among honey bees.

Keywords: *Apis mellifera*, tracheal mite infestation, population, diagnosis.

Citation: Hernández-Dionicio, E., Utrera-Quintana, F., Castillo-González, F., Otero-Colina, G. (2023). *Acarapis woodi* (Rennie), honey bee endoparasite: from the pest of the century to a forgotten case in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2506

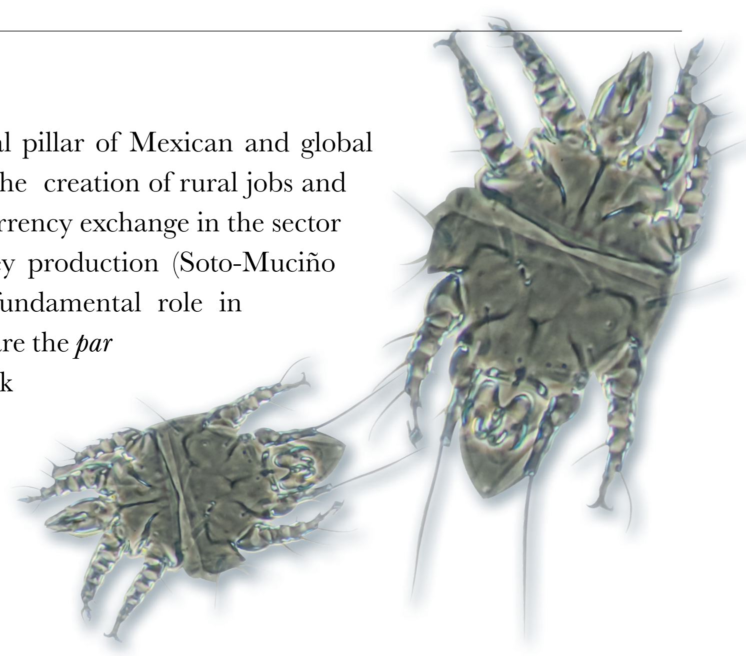
Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 04, 2023.
Accepted: June 19, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 69-78.

INTRODUCTION

Beekeeping is a fundamental pillar of Mexican and global agriculture, both as a result of the creation of rural jobs and as the third source of foreign currency exchange in the sector due to the high levels of honey production (Soto-Muciño *et al.*, 2017). Insects play a fundamental role in ecosystems as pollinators. Bees are the *par excellence* participants in this task and consequently they have enormous economic and ecological importance. Most of the food that



humans consume and trade directly or indirectly depends on pollination by bees (Pantoja *et al.*, 2014).

Despite their benefits, a significant reduction in the abundance of pollinators has been reported worldwide, as a result of environmental deterioration, intensive agriculture, the use of pesticides, the introduction of non-native species, diseases, and climate change (González-Varo *et al.*, 2013). Cereals that depend on anemophily and are part of the human diet (such as corn and wheat) may not be affected to such a degree by the decrease in bees. Nevertheless, forage crops used for meat and dairy production will undoubtedly be severely affected by pollinator decline (Spivak *et al.*, 2010).

The *Acarapis woodi* (Rennie) mite (Figure 1) is an obligate parasite of *Apis mellifera* L. (Pettis and Wilson, 1996) and *A. cerana* Fabricius (Maeda and Sakamoto, 2016) bees. It lives inside the tracheae of bees, especially the tracheal trunks (Figure 2), but can also be found in other parts of the respiratory system, including the air sacs (Wilson *et al.*, 1997). Its entire life cycle takes place within this microhabitat, but adult females can leave the bees in search of another specimen to parasitize, usually young adult bees less than four days old (Hoy, 2011). These mites feed by perforating the tracheae of bees and sucking the hemolymph that bathes them; as a result of their feeding, the tracheae turn dark and may even collapse (Figure 3) (Scott-Dupree and Otis, 1992).

In 1916 *A. woodi* was discovered in Scotland, UK (Bailey, 1999). A few years earlier, a pronounced mortality of bee colonies was recorded in the Isle of Wight, southern England, an event known as "the Isle of Wight disease" (Bailey, 1964). When *A. woodi* was found to be an endoparasite of bees, it was associated with and blamed for the disease. The mite was even said to have been discovered on the Isle of Wight, not in Scotland. The nature and intensity of the damage caused by *A. woodi* in bees have been the subject of much debate: some maintain that bee mortality in the Isle of Wight was caused by this mite (Adam,

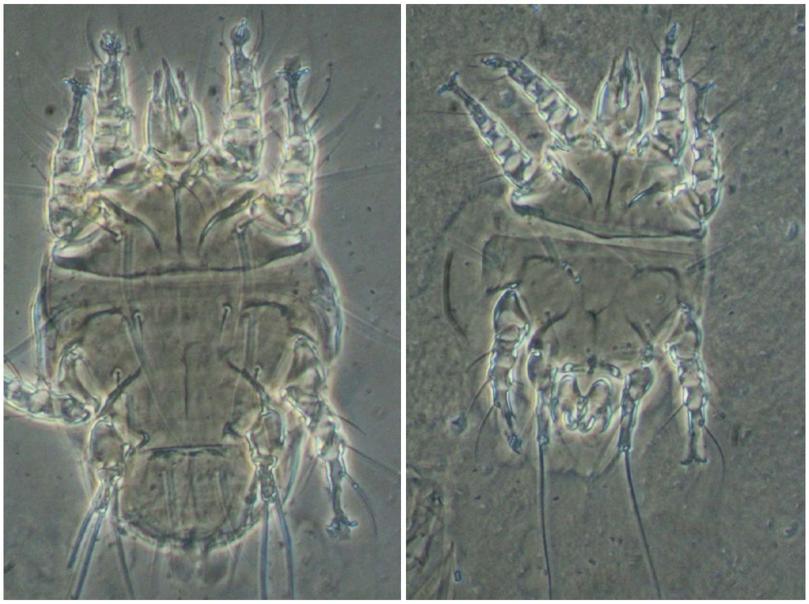


Figure 1. Photomicrographs of *Acarapis woodi*. Adult female, left. Adult male, right.

Figure 2. Tracheal trunk of a bee (*Apis mellifera*) filled with numerous specimens of *Acarapis woodi*.

Figure 3. Melanization of the trachea of bees (*Apis mellifera*) caused by the infestation by *Acarapis woodi*.

1968), while others (Bailey, 1958, 1961, 1964, 1999) argue that the most evident damage is the reduction in the longevity of adult bees and a greater mortality of colonies at the end of winter, when a high percentage of workers have been parasitized.

Despite Bailey's insistent arguments (1958, 1961, 1964, 1999), *A. woodi* was considered a major threat to beekeeping throughout the Americas. Consequently, in 1922 the United States of America restricted the import of bees from anywhere in Europe (Menapace and Wilson, 1980). In the years following its discovery, *A. woodi* spread to several countries in Europe, Africa, and South Asia (Morse, 1978), undoubtedly as a result of the transport of parasitized bees. However, the entire American continent was assumed to be free of its presence. Nevertheless, *A. woodi* was detected for the first time in the American continent in 1971, specifically in Brazil (Nascimento *et al.*, 1971). It was just a matter of time before it spread to neighboring countries.

The finding of *A. woodi* in the Americas caused alarm, given the obvious risk that it could spread both to the north and the south, and the consequent and significant damage that it would cause to bees in various countries. Researches were carried out to determine its northwards advance. As a result, it was discovered in 1980 in Colombia (Menapace and Wilson, 1980), in 1982 in Mexico (Wilson and Nunamaker, 1982), and in 1984 in the United States (Delfinado-Baker, 1984).

Based on the somewhat alarmist data of Adam (1968), Menapace and Wilson (1980), and others, the idea was posited that *A. woodi* would cause severe damage to bees in the American continent. Consequently, activities were conducted to assess its levels of infestation and damage, to determine if Africanized bees —which had recently reached Mexico— were carriers of the said mite, and to contain its spread.

Products were developed to control it (García Figueroa and Arechavaleta-Velasco, 2018). Among other relevant results, Romero-Vera and Otero-Colina (1992) determined that, although Africanized bees can indeed be parasitized by *A. woodi* (with infestation levels similar to those of European bees), the first migratory Africanized bees that reached North America were not carriers of said mite. Instead, they acquiried it from previously parasitized European bees in Mexico.

After initially fearing the presence of *A. woodi* in Mexico, beekeepers started to notice that the problem was not as serious as they had been led to believe (testimonial information collected by the authors). Since the diagnosis of the presence of *A. woodi* involved a very laborious method, it was rarely carried out by beekeepers; nevertheless, bee mortality comparable to that of the Isle of Wight disease and potentially associated with *A. woodi* parasitism was never observed. Those who did conduct a diagnosis noticed that, as the years went by, the infestation levels decreased and parasitized bees became rare (García Figueroa and Arechavaleta-Velasco, 2018).

Several hypotheses were put forward to explain the reduction in the intensity of bee parasitism by *A. woodi*: that the climate in tropical and subtropical areas, such as Mexico, is not favorable for the development and increase of their populations (Bailey, 1958); that applying acaricides to control the *V. destructor* mite, detected in Mexico in 1992 (Rodríguez-Dehaibes *et al*., 1992), had a collateral effect against *A. woodi* (Martínez-Puc *et al*., 2011); and that bees developed resistance to infestation by *A. woodi* (Pettis and Pankiw, 1998).

The aim of this work is to describe the current state of bee parasitism by *A. woodi* in Mexico and propose a hypothesis to explain the reduction in infestation levels. Information has been taken from numerous studies conducted throughout the years in which this mite has been present in the country and it was complemented with observations made in an apiary of Euro-African bee colonies in which no treatments against *V. destructor* have ever been applied.

MATERIALS AND METHODS

Study group

The research was carried out in February 2019 in the experimental apiary of the Posgrado en Recursos Genéticos y Productividad Genética, Colegio de Postgraduados - Campus Montecillo, Texcoco, Estado de México (19° 17' N and 98° 54' W; 2,240 m.a.s.l.), where n=20 bees were selected from each of 54 previously selected hives. The samples were dissected in the laboratory within two days after the collection.

Sample collection

The samples were collected in the morning (9:00 a.m.), when the foraging activity begins, to avoid stressing the bees. Adult bees were selected from the inside lid of the hive, the edge combs, and the entryway. When hives had stacked supers, bees were collected from the honey combs, the place where the older foraging workers can be found. The worker bees were put in wide-mouthed plastic jars with 30 mL of 70% ethyl alcohol, which caused their immediate death and enabled their short-term conservation. The samples were then taken to the Entomology and Acarology laboratory for diagnosis.

Dissection technique

The 20-bee sample was placed on a paper towel to remove the excess alcohol, whose aqueous consistency could hinder cutting during dissection. Another towel was placed on top of the sample for better absorption, applying light pressure by hand. The sample was left to rest for about 2 min. Meanwhile, five separate drops of lactic acid were put on a slide. Each bee was placed between the thumb and forefinger of the researchers, with its ventral area facing up and the head was removed with entomological tweezers (the first pair of legs usually come out with the head). Fine-tipped scissors were used to make a 2 to 3-mm thick cut parallel to the one that resulted from the removal of the head. The tracheae were left exposed along with the spiracles —which are the entry route for mites. Finally, a thorax slice was taken with the same entomological tweezers and placed on one of the five drops of lactic acid on the slide. This technique was repeated four times, with five bees per slide.

Microscopic observation

Once the thoracic muscles of the bees softened as an effect of lactic acid, a stereoscopic microscope was used to separate the thoracic tracheae at the closest point to the spiracle and two dissecting forceps were used to expose any mites that might be inside. Subsequently, the tracheae were mounted between slides with lactic acid as a preparation medium. The samples were observed using a phase contrast microscope with 100x and 400x magnifications. Previous preparations from the mite collection of the Laboratorio de Acarología of the Colegio de Postgraduados show that, even without stained material, this microscope provides a clear view of the mites inside the tracheae, as well as of the dark spots that result from the infestation by *A. woodi*.

RESULTS AND DISCUSSION

No bees from the examined colonies were infested by *A. woodi* (Table 1). This result agrees with the findings of Martínez-Puc *et al.* (2011) and Martínez-Cesáreo *et al.* (2016), who did not detect infestation by *A. woodi* mite in bee colonies from the states of Yucatán and Estado de México, respectively. For their part, García-Figueroa and Arechavaleta-Velasco (2018) estimated a 0.02% prevalence of infested bees in the state of Morelos. The

Table 1. Results of the dissection test carried out to observe mites in the tracheae of sampled bees.

Number	ID-Hive	Result	Number	ID-hive	Result
1	075	(−)	28	207	(−)
2	074	(−)	29	034	(−)
3	053	(−)	30	083	(−)
4	021	(−)	31	049	(−)
5	064	(−)	32	07	(−)
6	001	(−)	33	087	(−)
7	003	(−)	34	073	(−)
8	038	(−)	35	079	(−)
9	095	(−)	36	080	(−)
10	026	(−)	37	023	(−)
11	086	(−)	38	104	(−)
12	066	(−)	39	122	(−)
13	030	(−)	40	062	(−)
14	184B	(−)	41	155	(−)
15	084	(−)	42	069	(−)
16	04	(−)	43	209	(−)
17	018	(−)	44	052	(−)
18	150	(−)	45	024	(−)
19	070	(−)	46	092	(−)
20	013	(−)	47	119	(−)
21	060	(−)	48	048	(−)
22	09	(−)	49	08	(−)
23	06	(−)	50	036	(−)
24	081	(−)	51	187	(−)
25	056	(−)	52	122	(−)
26	112	(−)	53	020	(−)
27	015	(−)	54	019	(−)

ID-hive=hive identification; Number=sequence number; (−)=negative for mites.

absence of infestation by *A. woodi* or infestation levels so low that they are undetectable with the methods used seem to be a widespread phenomenon in sites with a tropical or subtropical climate in the Americas. For example, Calderón *et al.* (2007) detected this mite in 1.1% of the bees they examined in Costa Rica, while Peixoto *et al.* (2019) and Szawarski *et al.* (2017) did not detect it in Brazil and Argentina, respectively.

Numerical data for the infestation of bees by *A. woodi* reported by various authors almost one century after its discovery (Bailey, 1999) are difficult to compare, as a result of the wide differences in the techniques used to quantify parasitism. The method usually used to observe *A. woodi* has been to dissect the thorax of adult worker bees and view them under stereoscopic or biological microscopes. However, even that method could have variations. For example, Martínez Puc *et al.* (2011) only detached the head of the bees along with the first pair of legs, exposing the thoracic tracheae and, without further cuts, observed them frontwise with a stereoscopic microscope. Using a more refined method, Calderón *et al.* (2007) made a second cut after they detached the head and the first pair of legs to obtain an approximately 2-mm thick thorax segment. They placed that segment in 10% potassium hydroxide to macerate the muscles, separated the thoracic tracheae to make a more detailed observation, and even counted the *A. woodi* specimens.

Since dissecting the thoracic discs for observation involves a very laborious method, the number of bees examined per hive is usually low. It is hard to detect mild infestations with samples of 10 (Wilson and Nunamaker, 1982) or 30 specimens (Calderón *et al.*, 2007).

Other methods based on DNA analysis have been recently developed to detect *A. woodi* in bees (Garrido-Bailón *et al.*, 2012; Pietropaoli *et al.*, 2022). These methods for the observation of the interior of the tracheae of the bees are more sensitive and less laborious than those involving the dissection of the specimens. However, they can only attest to the presence or absence of *A. woodi* in the samples.

The values obtained from the above-mentioned methods may also vary. Scientists usually estimate only the percentage of infested bees (Calderón *et al.*, 2007). Others estimate infestation levels according to the number of specimens found in the tracheae (Otis, 1986; Romero-Vera and Otero-Colina, 1992). Still others look for mites in one or both tracheae (Bailey, 1958). However, specifically in the American continent (and more precisely in Mexico), all values obtained from the diagnostic methods indicate a net reduction in *A. woodi* infestation compared to the first years after the detection of this mite in the country. Back then, Eischen *et al.* (1990) estimated that 60% of the colonies were infested, while Romero-Vera and Otero-Colina estimated an almost 100% infestation and comparable levels of severity (number of *A. woodi* specimens in individual bees) among Africanized and European bees. These results contrast both with the most recent data (Marínez Puc *et al.*, 2011; Martínez-Cesáreo *et al.*, 2016) and with this research.

Although *A. woodi* infestation has ostensibly decreased up to the point where it has almost disappeared, several hypotheses haven been posited to explain this phenomenon. One of them is that the climate in Mexico is not favorable for the development of high mite populations (Bailey, 1958). However, this is a questionable proposal, since Mexico has a wide variety of climates and more than one scenario must be taken into consideration. The works of Eischen *et al.* (1990) and Romero-Vera and Otero-Colina (1992) stand out among the first instances in which *A. woodi* was detected in Mexico. The former observed 60% of infested colonies (40% of them with ≥30% infested workers) in the states of Nuevo León and Tamaulipas, while the latter detected approximately 10% of bees severely infested with *A. woodi* (more than 15 mites in their tracheae) in 515 of 516 hives in the states of Chiapas, Quintana Roo, and Tabasco.

The second hypothesis postulates that the pesticides used to control *V. destructor* —which was detected in Mexico in 1992 (Rodríguez-Dehaibes *et al.*, 1992)— can also control *A. woodi* (Martínez Puc *et al.*, 2011; García-Figueroa and Arechavaleta-Velasco, 2018). No tests have been conducted to determine the effect of these pesticides on *A. woodi*, most likely because this mite became increasingly difficult to detect and no obvious damage was observed that could be associated with its parasitism. However, this hypothesis ignores that the control of *V. destructor* was never widespread. Many beekeepers never treated their hives against this mite and wild colonies —whose numbers are unknown, but which undoubtedly exist— were not treated against *V. destructor* and therefore would have served as *A. woodi* reservoirs and sources of contagion. The results of this work confirm past reports: the sampled colonies have never been treated against *V. destructor*.

Therefore, the most feasible hypothesis is that bees developed resistance to *A. woodi* over the years. This hypothesis is particularly suitable in the case of Mexico, but does not rule out that other hypotheses can be valid to a lesser extent, since they are not mutually exclusive. During the initial stages of the establishment of *A. woodi* in Mexico, the percentage of infested bees and infestation levels were high (Eischen *et al*., 1990; Romero-Vera and Otero-Colina, 1992), but these figures have decreased over the years. These results would confirm that bees with some degree of resistance gradually replaced vulnerable bees.

Determining the resistance mechanism against the potential presence of *A. woodi* in Mexico would be difficult, mainly given the almost complete lack of biological material infested by *A. woodi* that could be compared with healthy samples. Bees groom each other (Pettis and Pankiw, 1998) and perhaps this practice could be a valid defense mechanism in the case of Mexico. A physical barrier —such as setae or the size of the spiracles— might prevent mites from entering the tracheae of bees. As stated in the title of this paper, the much-feared entry of *A. woodi* into Mexico became a practically forgotten topic. However, this threat should not be overlooked, because once an organism develops resistance to a parasite, the parasite also evolves to evade such resistance —a well-known fact in the field of parasitology, particularly in the study of bee parasites (Cobey, 1997).

CONCLUSIONS

The infestation of bees by the *A. woodi* tracheal mite shows a decreasing trend, both in the material studied and in Mexico and other American countries. The cause of this phenomenon seems to be the resistance developed by bees.

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AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Effects of different VIUSID Agro® concentrations on the growth of *Coffea arabica* L. seedlings

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ABSTRACT

Objective: The VIUSID Agro® biostimulant contains amino acids, vitamins, and minerals. It was subjected to a biocatalytic process of molecular activation to improve its biological activity. In Cuba, its benefits have been demonstrated mainly in vegetables, sugar cane, and tobacco.

Design/Methodology/Approach: The effect of the product on the morpho-agronomic traits of coffee seedlings was evaluated through experiments carried out in the nursery of the Instituto de Investigaciones Agro-Forestales of Tercer Frente, from December 2019 to July 2020 and from October 2020 to June 2021, using a saran shade cloth. In a completely randomized design, 6 concentrations of VIUSID Agro® (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mL L^{-1} of water) were applied monthly on the second to the fifth pair of leaves. Seedling height, stem diameter, dry mass, leaf area, quality index, and efficiency were evaluated. Data were processed using a simple classification analysis of variance. The means were compared with Duncan's test. The concentrations and the leaf area were adjusted to several functions and the one with the highest coefficient of determination was selected.

Results: Coffee seedlings had a positive response to the biostimulant. The monthly foliar applications of VIUSID Agro® between the second and the fifth pair of leaves increased the morpho agronomic indicators of the seedlings.

Findings/Conclusions: VIUSID Agro® in concentrations ranging between 0.64 and 0.71mL L^{-1} of water caused significant increases in the leaf area of the seedlings.

Keywords: biostimulant, coffee tree, dose, growth promoter, nursery.

Citation: Bustamante-González, C. A., Vázquez-Osorio, Y., Fernández-Rosales, I., & Ferrás-Negrín, Y. (2023). Effects of different VIUSID Agro® concentrations on the growth of *Coffea arabica* L. seedlings. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2522

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 19, 2023.
Accepted: June 27, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 79-87.

INTRODUCTION

To increase crop production, several industries have developed nutritional complexes that contain micronutrients, amino acids, and plant extracts. These complexes have been called growth promoters or biostimulants [1].

In Cuba, the positive effect of biofertilizers and biostimulants on carrot (*Daucus carota* L.) [2], bean (*Phaseolus vulgaris* L.) [3], tomato (*Solanum lycopersicum* L.) [4], and soybean (*Glycine max* L.) [5] has been demonstrated. When the effect of FitoMas E on passion fruit

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(*Passiflora edullis* L.) was studied [6], the doses were found to have a similar effect on the variables analyzed and it was considered as a sign that an adequate concentration of the biostimulant promotes positive changes in the metabolism and physiology of plants and uniformly improves their agronomic traits.

When studying the effect of increasing doses of Enerplant in lettuce (*Lactuca sativa* L.), low concentrations of this product were found to induce organ growth, although not their number [7].

Likewise, when the effect of 6 doses of a biostimulant derived from algae in wine grape (*Vitis vinifera* L.) were analyzed, the best results were obtained with the 0.75 mL dose; however, the use of higher doses reduced the evaluated variables [8]. In the case of passion fruit, increasing doses of the Stimulate biostimulant on the leaf area had no effect [9].

Cuban researchers have studied the use of bioproducts in coffee (*Coffea arabica* L.) [10]. Increases in seedling height were reported in a range of 44 to 73% as a result of the application of a 250 mL L^{-1} m^2 concentration of FitoMas E [11], while its application in 0.2% concentrations led to 37% increases of this indicator with respect to the control [12].

VIUSID Agro® is also registered as one of the formulations used as a plant growth stimulant in Cuba. This product essentially contains amino acids, vitamins, and minerals and was subjected to a biocatalytic process of molecular activation to improve its biological activity and the biochemical reactivity of all its molecules. This process favored the vegetative and reproductive stage of the crops and increased the stem length and the number of leaves, flowers, and fruits, which had a positive influence on yields [1].

In Cuba, the positive effect of the foliar application of VIUSID Agro® has been demonstrated in *Solanum lycopersicum* L. [1], beans, radish (*Raphanus sativus* L.), *Beta vulgaris* L., lettuce [13], *Colocasia esculenta* Schott [14], *Nicotiana tabacum* L. [15], and *Saccharum* spp. [16]. In Mexico, productive increases were achieved in winter squash (*Cucurbita argyrosperma* Huber) [17]. Meanwhile, 2.0 and 4.0 mL doses of VIUSID Agro® dissolved in 5 L of water promoted highly significant results: more fruits per coffee plant, greater equatorial and longitudinal diameter of fruits, and higher yield per plant and per hectare of coffee than the control treatment [18].

The production of coffee seedlings is a vital task for the development of coffee growing in Cuba. Therefore, techniques that produce very highquality plants are required to ensure the durability of the plantations.

No information is available regarding the use of VIUSID Agro® in the production of coffee seedlings. Consequently, this research was carried out to study the effect of different biostimulant concentrations in the morpho-agronomic traits of coffee.

MATERIALS AND METHODS

The experiments were carried out in the nursery of the Instituto de Investigaciones Agro-Forestales, Unidad de Ciencia y Tecnología de Base - Tercer Frente (20° 08' 11.06" N y 76° 16' 22.27" W) located in the Consejo Popular Cruce de Los Baños, Tercer Frente municipality. The experiments took place in two periods: the first from December 2019 to July 2020 and the second from October 2020 to June 2021.

The experiment aimed to study the effect of 6 concentrations of VIUSID Agro® on the growth of *Coffea arabica* L. seedlings. In a completely randomized design, the following concentrations were studied: Without VIUSID Agro® (0 ml L^{-1}) - control; 0.2 ml L^{-1} of VIUSID Agro®; 0.4 ml L^{-1} of VIUSID Agro®; 0.6 ml L^{-1} of VIUSID Agro® 0.8 ml L^{-1} of VIUSID Agro®; 1.0 ml L^{-1} of VIUSID Agro®.

During the experimental period, VIUSID Agro® was applied monthly from the second to the fifth pair of leaves, for a total of 4 applications. The product was applied with a 16 L Matabi manual backpack sprayer, in the early hours of the morning. The control treatment was sprayed with water. To attenuate the sun effect, a saran shade cloth was used to regulate the shade to 60%.

Two "Isla 6-14" variety coffee seeds were sown in 2.5 cm × 25 cm polyethylene bags, which were filled with a 3:1 ratio of brown soil-filter cake substrate. During both years of the experimental period, the average temperature was around 24 °C, while the accumulated rainfall for 2020 and 2021 was 626 mm and 1,221 mm, respectively, mainly as a consequence of the rains in October and November (Figure 1).

Each treatment consisted of 45 seedlings arranged in 3 rows (15 seedlings per row). The following variables were evaluated in 10 plants per treatment:

- Plant height (cm), measured from the base of the stem to the apical meristem with a graduated ruler.
- Stem diameter (mm), measured with a Dijite brand digital caliper one cm above the soil.
- Leaf area (cm^2), estimated by the measurement of the linear dimensions of the leaves according to the following formula:

$$AF\left(\text{cm}^2\right) = length \times width \times 0.64 \quad [19].$$

- Aerial and root dry mass (g). Plants were divided by organs (leaves, stems, and roots) and washed with water, placed on paper, and dried in a forced-air oven at 70 °C until constant weight was achieved.

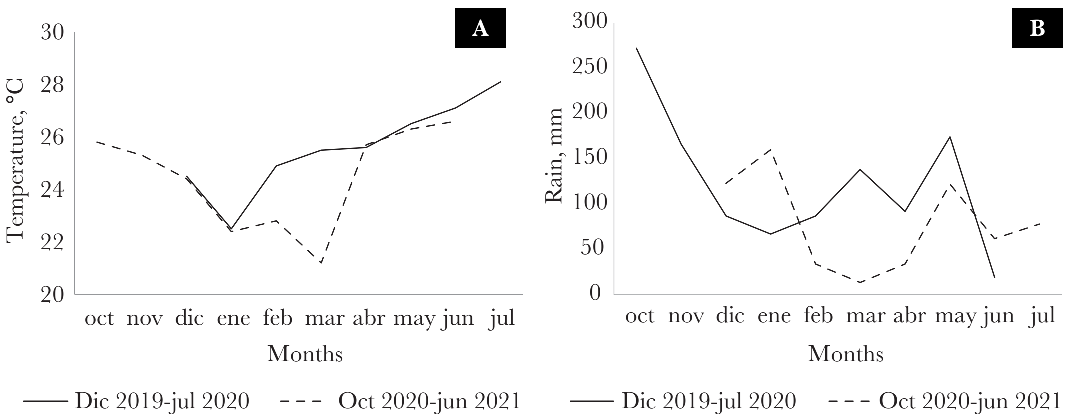

Figure 1. A: Monthly temperature and B: rainfall during the experimental period.

- Quality index [20].
- Efficiency index [21], calculated for the variables evaluated in the experiment, based on the following formula:

$$Efficiency\ index = \left(\frac{treatment\ value\ c - control\ value}{control\ value}\right) \times 100$$

The simple classification analysis of variance was carried out using a completely randomized design, according to the fixed-effects linear model. The data was processed using the Statistica software for Windows. The normality of the data and the homogeneity of the variance were verified by the Kolmogorov-Smirnov test and by the Levene test, respectively. Duncan's multiple-range test ($p \leq 0.05\%$) was used to determine the differences between the treatments.

To recommend the biostimulant concentration, the data of the relative increase in leaf area were correlated with the different concentrations and the function with the highest coefficient of determination was selected.

RESULTS AND DISCUSSION

Coffee seedling height

Plant height is a trait that is strongly modified by environmental conditions. Humidity, mineral nutrition, and light intensity are some of the factors that exercise the greatest influence on it.

VIUSID Agro® concentrations had a positive effect on height (Table 1). In 2020, this variable gradually increased until it reached a concentration of 0.4 mL L^{-1}; afterwards, a significant decrease trend was recorded. The greatest growth with respect to the control (28.9%) was found with the application of the 0.8 mL L^{-1} concentration, although it was not statistically different from the growth achieved with 0.4 mL L^{-1}.

In 2021, a positive result (like that of 2020) was found regarding the increase of the VIUSID Agro® and the coffee seedling height. A tendency towards an almost linear height growth with increasing concentrations was observed (Table 1). The action of 0.2-0.6

Table 1. Effect of VIUSID Agro® concentrations on the height and diameter stem of coffee seedlings.

Concentration (mL L^{-1})	Height (cm)		Stem diameter (mm)	
	2020	2021	2020	2021
0.0	16.30 d	21.07 d	2.82 bc	3.37 b
0.2	18.50 c	23.14 c	2.65 c	3.35 b
0.4	20.07ab	23.75 c	2.89 abc	3.39 b
0.6	19.53 b	22.87 c	2.99 ab	3.38 b
0.8	21.02 a	26.35 a	3.10 a	3.75 a
1.0	17.58 cd	25.30 b	2.73 c	3.51 b
E, E, $\bar{x}$	0.20*	0.31*	0.04	0.08*

* Means with different letters in the same column differ from each other according to Duncan's test for $p<0.05$.

concentrations was statistically similar and caused a 9.8-8.50% increase in this indicator. The greatest increase (25%) in this indicator with respect to the control was found with the 0.8 mL L^{-1} concentration of VIUSID Agro®.

As a consequence of the application of 250 mL L^{-1} m^2 of FitoMas E, a 44-73% increases in coffee seedling height have also been recorded [11].

Stem diameter

In both years, the highest absolute value of the stem diameter was reached with the application of 0.8 mL L^{-1} (Table 1) and it was significantly different from the control. The increase in this indicator was lower than the increase in height and it amounted to 10% and 12%, in 2020 and 2021, respectively.

Dry mass

Biomass is considered an important indicator of the ecological and management processes that occur in the vegetation and it reflects the conditions of the site and the soil, water, and solar radiation resources available in it [22].

The application of a 0.6-mL L^{-1} concentration of VIUSID Agro® in 2020 increased the total dry mass by 17% with respect to the control, although no significant difference was found with the action of the biostimulant in the 0.4 and 0.8 mL L^{-1} concentrations (Table 2).

The effect of the biostimulant on the dry mass of the leaves in 2020 did not show a consistent pattern (Table 2). For the stem dry mass, the concentrations increased this variable, although no differences were found between them. The application of 0.4, 0.6, and 0.8 mL L^{-1} concentrations of VIUSID Agro® significantly increased the dry mass of the root with respect to the control and the 0.2 mL concentration.

The ideal balance for the growth of the different plant organs is variable: a certain endogenous concentration can favor the growth of one organ and inhibit the growth of another [23]. This last behavior was observed in these experiments during the evaluation of the response of coffee seedlings to the biostimulant.

Table 2. Effect of VIUSID Agro® concentrations on the dry mass of coffee seedlings and their composition (2020).

Concentration (mL L^{-1})	Dry matter (g)			
	Leaf	**Stem**	**Root**	**Total**
0.0	1.75 ab	0.28 b	0.44 b	2.47 cd
0.2	1.50 b	0.36 a	0.48 b	2.34 d
0.4	1.60 ab	0.36 a	0.66 a	2.62 abc
0.6	1.84[a]	0.36 a	0.62 a	2.82 a
0.8	1.77 a	0.41 a	0.60 a	2.78 ab
1.0	1.67 ab	0.37 a	0.50 b	2.54 bcd
E, E, $\bar{x}$	0.03*	0.01*	0.01*	0.04*

* Means with different letters in the same column differ from each other according to Duncan's test for $p<0.05$.

During the evaluation of the effect of the concentrations on the seedlings dry mass in 2021 (Table 3), the application of 0.8 mL L^{-1} of VIUSID Agro® was found to significantly increase this variable, unlike the rest of the treatments (98.4% with respect to the control).

Quality index

With the application of increasing doses of VIUSID Agro® in 2020, the quality index of the seedlings tended to increase as well, with similar values for the 0.4, 0.6, and 0.8 mL L^{-1} concentrations. The 1 mL L^{-1} concentration of the biostimulant resulted in values similar to those of the control treatment. In 2021, this indicator recorded the highest value with the application of 0.8 mL, which differed from the rest of the treatments (Table 4).

Leaf area

Leaf area is an important variable in most agricultural and physiological studies about plant growth, light uptake, photosynthetic efficiency, respiration, transpiration, response to irrigation and fertilization, nutrient use, and especially carbon assimilation during the plant life cycle [24-26].

The leaf area is an index that adequately expresses the response of the integrated growth of the coffee seedlings and has higher quantitative values than the other variables [27,28].

Table 3. Effect of VIUSID Agro® concentrations on the dry mass of coffee seedlings and their composition (2021).

Concentration (mL L^{-1})	Dry matter (g)			
	Leaf	Stem	Root	Total
0.0	1.75 c	0.46 d	0.52 e	2.73 d
0.2	2.473 b	0.90 b	0.93 b	4.39 b
0.4	2.58 b	1.10 a	0.81 c	4.49 b
0.6	2.57 b	0.75 bc	0.99 b	4.31 b
0.8	3.12 a	1.20 a	1.11 a	5.43 a
1.0	2.47 b	0.63 c	0.67 d	3.77 c
E, E, $\bar{x}$	0.074*	0.046*	0.032*	0.13*

*Means with different letters differ by $p \leq 0.05$ according to Duncan's test.

Table 4. Effect of VIUSID Agro® concentrations on the quality index and leaf area of coffee seedlings.

Concentration (mL L^{-1})	2020		2021	
	Quality index	Leaf area (cm^2)	Quality index	Leaf area (cm^2)
0	0.24 c	305.37 b	0.26 d	402.7 d
0.2	0.22 c	298.42 b	0.41 b	409.2 d
0.4	0.27 ab	388.62 a	0.38 b	453.0 c
0.6	0.28 a	396.26 a	0.42 b	461.7 bc
0.8	0.27 ab	403.05 a	0.52 a	528.0 a
1.0	0.24 bc	317.54 b	0.31 c	487.2 b
E, E, $\bar{x}$	0.004*	7.42*	0.015*	10.85*

*Means with different letters differ by $p \leq 0.05$ according to Duncan's test.

In 2020, the VIUSID Agro® concentrations significantly increased the leaf area of the coffee seedlings, starting from the 0.4 mL L^{-1} concentration. This increase was 27% higher than the control (Table 4) and did not differ from the effect achieved with concentrations of up to 0.8 mL. When applying a 1 mL L^{-1} concentration of VIUSID Agro®, the leaf area decreased significantly reaching a statistically similar value to the control.

In 2021, a similar increase in leaf area was recorded after the application of VIUSID Agro® (Table 4). The highest average value was found when a concentration of 0.8 ml L^{-1} of the biostimulant was applied, causing a 31% increase with respect to the control; this result significantly differed from the rest of the treatments. A decrease in this variable was also found when the highest concentration was applied.

VIUSID Agro® had a positive effect on the growth of tobacco seedlings and increased the fresh mass, dry mass, and the stem diameter by 28.4%, 30.5%, and 41.2%, respectively. Therefore, the product should be applied in doses of 0.5, 0.7, and 1.0 L ha^{-1} [15]. The correlation of the relative increases in the leaf area with the concentrations of the biostimulant showed the greatest adjustments to the quadratic functions, recording maximum values of 0.64 (2020) and 0.71 (2021) (Figure 2). Therefore, the use of VIUSID Agro® can be recommended as a biostimulant for Cuba's coffee seedlings production.

The results of the biostimulant application can be attributed to its composition: Its formula includes potassium phosphate (which favors the formation of carbohydrates and is necessary for the transfer and storage of energy in plants [29]), zinc sulfate (which favors the formation and development of new tissues), and glycine (which is a vital amino acid for the growth process and photorespiration).

Another equally important component is folic acid —an important coenzyme in the metabolism of amino acids and in the synthesis of the nitrogenous bases required for the formation of new tissues.

VIUSID Agro® and other products with amino acids [29] have three types of effects on the plants. One of these effects is hormonal: when amino acids enter the plants, they stimulate the formation of chlorophyll and indoleacetic acid (IAA), as well as the production of vitamins and the synthesis of numerous enzymatic systems [30].

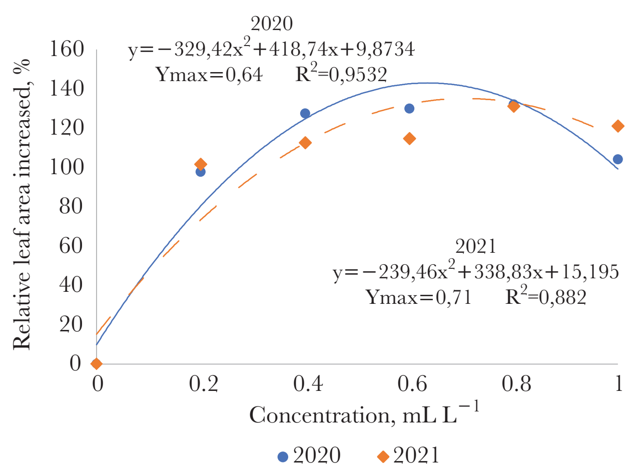

Figure 2. Relative increase in leaf area due to the effect of VIUSID Agro® concentrations.

Amino acids are stimulants that allow the plant to "save energy" and catalyze the synthesis of sugars, starch, and other components of leaves, flowers, and fruits. They contribute to the increase of chlorophyll in the leaves and delay aging, thereby intensifying the performance of photosynthesis [18].

CONCLUSIONS

The monthly application of VIUSID Agro® between the second and fifth pair of leaves favored the morpho-agronomic indicators of coffee seedlings. The VIUSID Agro® in 0.64 to 0.71 mL L^{-1} concentrations of water favored the greatest increases in the leaf area of the coffee seedlings.

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Colegio de Postgraduados

Effects of the combining ability on *mirador* chili (*Capsicum annuum* L.) populations native to Veracruz, Mexico

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ABSTRACT
Objective: To evaluate the effects of the general and specific combining abilities on the agronomic traits of the native populations of *mirador* chili and to identify potential genotypes for the genetic improvement of this crop.
Design/Methodology/Approach: The general and specific combining abilities were calculated to determine the agronomic traits and yield of five *mirador* chili populations, using Griffing's method 2, model I, which includes parental lines and direct crosses.
Results: The P1, P4, and P3 genotypes recorded the highest and positive values. They also recorded a significative difference regarding the general combining ability. Meanwhile, the P1×P5 and P4×P5 crosses recorded the highest specific combining ability values.
Study Limitations/Implications: We were not able to establish diverse evaluation environments and genomic selection studies using molecular markers.
Findings/Conclusions: Information about the yield potential of five *mirador* chili was generated. These results are important for the development of a genetic improvement program and for the selection of the method that will be used.

Keywords: genetic improvement, native populations, yield.

Citation: López-Benítez, A., Gayosso-Barragán, O., Alcalá-Rico, J. S.G.J., Chávez-Aguilar, G. & Martínez-Osorio, A. (2023). Effects of the combining ability on mirador chili (*Capsicum annuum* L.) populations native to Veracruz, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i6.2528

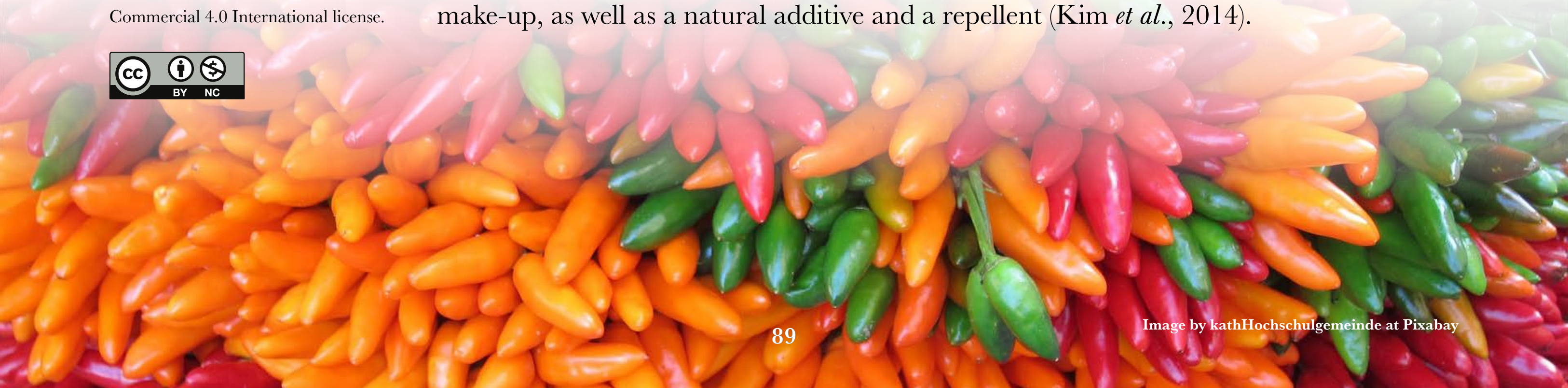
Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 22, 2023.
Accepted: June 21, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 89-96.

INTRODUCTION

Chili is an important crop worldwide, grown in tropical and subtropical areas. The genus *Capsicum* has a wide genetic diversity. It is made up of 38 species, including several worldwide domesticated species, such as *C. annuum*, *C. frutescens*, *C. baccatum*, *C. chinense*, and *C. pubescens* (Csilléry, 2006). Regarding its nutritional value, chili is an excellent source of essential vitamins, minerals, and nutrients, which make a major contribution to human health (Gupta *et al*., 2019). Additionally, chili is used to manufacture pharmaceuticals and make-up, as well as a natural additive and a repellent (Kim *et al*., 2014).



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Mexico is the center of origin, domestication, and diversification of *Capsicum annuum* L.; no other species is grown, produced, commercialized, and consumed more around the world (Kraft *et al.*, 2014). The importance of this crop in Mexico is the result of its great diversity of shape, color, flavor, aroma, and uses. For over 8,000 years, *Capsicum annuum* L. has been subjected to different selection and domestication processes by different human populations, using both wild and domesticated varieties, from the different subregions of Central and South America, including Mexico and Mesoamerica (Perry and Flannery, 2007). Different species and varieties of chili are grown in Mexico, including ancho, jalapeño, serrano, poblano, sweet red pepper, and *mirador*. The last chili is native to the middle Huasteca region of Veracruz, where it is grown in small areas, as monocrop or associated with corn. The plants are medium-sized (60-130 cm height). The plants bear erect or pendulous fruits that are 2.5-6.0 cm long and 0.6-2.0 cm wide (Ramírez *et al.*, 2018, Martínez, 2011).

The use of hybrid varieties is common among producers, as a result of their high yield (Muthumanickam and Anburani, 2017); consequently, the selection of inbred lines is a major step in an improvement program, because there is a huge opportunity to improve yield and fruit quality (Singh *et al.*, 2014). During the selection process of the inbred lines, the identification of heterotic groups and the analysis of the combining ability play an important role in chili cultivation improvement, allowing the selection of specimens with features of interest and a wide genetic variability (Hallauer and Miranda, 1981).

The combining ability of a genotype is its capacity to transfer a higher yield trait to its crosses (Thilak *et al.*, 2019). There are two types of combining abilities: general combining ability (GCA) and specific combining ability (SCA). Even before the beginning of the process, these two abilities are part of the genetic improvement. The GCA is mainly the consequence of the additive genetic action, while the SCA is the result of the non-additive genetic action (Singh, 2015). The analysis of the GCA enables the appropriate identification of the parental lines that can transmit their desirable characteristics. Meanwhile, the SCA allows to determine the outstanding F1 hybrid combinations, resulting from crosses between varieties or lines. Likewise, this type of analysis provides information about the type of genetic action that determines the expression of a characteristic, which is fundamental to identify which improvement method will be used.

The studies about GCA and SCA of the inbred lines have shown that the nature of the genetic action in the expression of a particular characteristic helps to identify the best crosses based on a parental line combination (De Sá Mendes *et al.*, 2019).

The objective of this study was to determine the general and specific combining abilities of agronomic traits in *mirador* chili native varieties and to identify potential genotypes for future genetic improvement programs.

MATERIALS AND METHODS

Vegetal material

Five *mirador* chili populations were collected in the state of Veracruz. Subsequently, along with their ten F1 potentially direct crosses, they were evaluated, using a diallel design, which involves their parents (Griffing's Method 2 (1956)). In order to carry out the hybridization process, the seeds were placed in 200-cell polystyrene boxes, filled with Peat-Moss commercial substratum (one seed per cavity). Forty days after the germination, the seedlings were planted in 4-L pots, following the regular agronomic handling recommended for this crop (Barrentes, 2010). They were placed under greenhouse conditions in the Universidad Autónoma Agraria Antonio Narro, in Buenavista, Saltillo, Coahuilla, Mexico (25° 21' 18" N and 101° 04' 48" W), at 1,781 m.a.s.l. Before the hybridization took place, the female flowers were emasculated and the pollen was extracted from the male flowers and carefully released on the stigma. To avoid cross-pollination, the female flowers were covered after the hybridization. Each cross was labelled with the name of the genotypes involved in the hybridization and the date in which it took place.

Establishment of the experiment in the field

Fifteen genotypes (parents and F1 crosses) were planted in a field using a random block experimental design, with four replicates. This procedure took place after the seedlings developed three or four true leaves and reached a height of 25 cm. The field was located in the Campo Experimental Las Huastecas of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) (22° 33' 59" N and 98° 09' 45" W), at 18 m.a.s.l. The experimental unit included five plants per 2-m long furrow, with 90 cm of separation between the furrows and 30 cm between plants.

Evaluated variables

Five plants with complete competence were evaluated per parent and cross. The following elements were recorded: days to flowering (DF), days to harvest (DH), plant height (PH), fruit diameter (FD), fruit length (FL), number of fruits (NF), and fruit weight (FW). DF was measured as the days from the transplant until 50 % of the plants blossomed (flowering stage). DH was the time when fruits reached their commercial ripeness. PH was measured from the base of the stem to the tallest part of the plant. The value of FD and FL was measured in cm. Finally, NF and FW were determined.

Genetic model

In order to calculate the general and specific combining abilities (GCA and SCA), the Griffing's method 2, model I was used (Griffing, 1956). This method includes parents and direct crosses, with $\left[p(p+1)\right]/2$ combinations. The following analytic model was used for the combining ability:

$$X_{ijk} = \mu + g_i + g_j + s_{ij} + b_k + (gb)_{ijk} + 1/bc\sum\sum e_{ijk}$$

Where: X_{ijk} = observed phenolic value; μ = general experimental mean; g_i and g_j = GCA effect of the parents; s_{ij} = SCA effect of the $i \times j$ ($s_{ij} = s_{ji}$) crosses; b_k = k block effect; $(gb)_{ijk}$ = effect of the interaction between the ij genotype and the k block; $1/bc\sum\sum e_{ijk}$ = residual effect of ijk.

Statistical analysis

The Dialel-SAS (Zhang and Kang, 2003) procedure of the SAS v. 9.0 statistical software was used to analyze the genetic effects of the GCA and the SCA.

RESULTS AND DISCUSSION

Diallel analysis

Highly significant differences ($p \leq 0.01$) were recorded regarding the genotypes and the general and specific combining abilities in all the evaluated characteristics (Table 1). The statistical difference shown by the genotypes can be a consequence of the genetic diversity of the evaluated populations. This difference leads to the identification of crosses with significative high yields. This result is important to measure the behavior of the agronomic traits in response to the hybrid combinations.

The statistical difference recorded for the GCA and SCA effects shows the importance of the additive and non-additive genetic effects in the evaluated variables, enabling the identification of those parental lines with the ability to transmit the desirable characteristics and the establishment of those outstanding F1 hybrid combinations. In addition, these values are an efficient tool for the appropriate selection of the improvement method to be used (Reyes *et al*., 2004).

Chakrabarty *et al.* (2019), Rodrigues *et al.* (2012), and Silva *et al.* (2017) recorded significative differences regarding the effect of GCA and SCA in the agronomic variables, using different chili parents and diverse evaluation environments. For their part, Ramesh *et al.* (2013) registered significatively high GCA and SCA values in chili, regarding the following characteristics: days to flowering, total yield, fruit length, fruit weight, plant height, pericarp thickness, number of seeds per fruit, and weight of 1,000 seeds. Meanwhile, Jindal *et al.* (2015) recorded significatively positive GCA and SCA dry matter, ascorbic acid, and capsaicin content. Finally, Rodrigues *et al.* (2012) recorded that fruit length, days to flowering, yield, number of fruits per plant, number of seeds per fruit, and fruit average weight had a significatively desirable GCA.

Effects of the general combining ability (GCA)

Table 2 shows the effects of the general combining ability in the fruit weight variable; the P1, P4, and P3 parents had the highest and positive values, with a significative

Table 1. Mean squares of the diallel analysis used to determine the yield characteristics of five *mirador* chili populations and their ten crosses.

Variation sources	Degree of freedom	Days to flowering	Days to harvest	Plant height	Fruit diameter	Fruit length	Number of fruits	Fruit weight
Repetitions	3	8.81 ns	19.39 ns	17.5 3 ns	0.01 ns	0.11 ns	631.51 ns	0.02 ns
Genotypes	14	329.34**	234.82**	190.03**	0.65**	1.03**	80682.7**	4.76**
GCA	4	93.12**	97.75**	196.02**	0.59**	0.95**	52451.58**	1.67**
SCA	9	423.83**	289.65**	187.63**	0.67**	1.06**	91975.19**	5.99**
R^2		95%	91%	57%	90%	75%	77%	70%

GCA (ACG): general combining ability; SCA (ACE): specific combining ability; *, ** Significative at 0.05 and 0.01 of probability, respectively, ns: not significative.

difference: 0.28, 0.06, and 0.05, respectively. P1 also obtained a positive and significative GCA regarding the diameter (0.08) and fruit length (0.31) variables. Likewise, P6 recorded positive and significative values for the fruit diameter variable (0.16). According to Zewdie and Bosland (2000), the GCA positive values can be understood as an expression of the variability found in the parents, which can be transmitted to their lineage.

In this study, P1 recorded outstanding characteristics, which can be taken into account as a good germplasm source for future improvement programs, as a result of the positive GCA values found in the fruit weight, plant height, fruit diameter, and fruit length variables. On the one hand, a low GCA value shows that the average of a parent in the cross with another parent does not vary much from the general average of the cross. On the other hand, a high GCA value shows that the average of the parents is higher or lower than the general average. Consequently, there is a strong proof of a desirable gene flux from parents to sons. This information about the concentration of mainly additive genes is highly relevant (Franco *et al.*, 2001). A high estimation of GCA indicates a higher inheritability and less environmental effects. It also can result in less genetic interactions and a higher selection gain (Topal *et al.*, 2004; Chigeza *et al.*, 2014).

On the one hand, a parent with outstanding agronomic traits may not necessarily produce the best hybrids during the hybridization process; on the other hand, if the other parent is appropriately selected, a parent with few outstanding agronomic traits can generate an appropriate combination (Bao *et al.*, 2009; Shukla and Pandey, 2008; Tyagi and Lal, 2005). Pessoa *et al.* (2021), Pech *et al.* (2010), and Hernández *et al.* (2021) recorded highly positive GCA values in the yield variables of several chili varieties. They also selected the genotypes with the highest potential to start an improvement program.

Effects of the specific combining ability (SCA)

According to Griffing (1956), the high absolute values of SCA indicate which parents were better or had a higher average than the GCA of both parents. In this study, the highest SCA values were recorded for the fruit weight variable, with the 1×5 and the 4×5 crosses, obtaining 1.61 and 1.36 values, respectively (Table 3). The specific combining ability reveals the best cross combination between genotypes for the development of hybrids with the desired characteristics. The results show that the right combinations can express those characteristics. The crosses with the highest fruit weight values can be the result of the additive effects of both parents and the interaction of the dominant alleles of one parent

Table 2. Effects of the general combining ability (GCA) on the agronomic traits of five parents of *mirador* chili.

Genotypes	Days to flowering	Days to harvest	Plant height	Fruit diameter	Fruit length	Number of fruits	Fruit weight
P1	0.53	−0.57	3.77	0.08*	0.31*	−20.67	0.28*
P2	1.53	2.20	0.74	−0.11	−0.12	13.71	−0.01
P3	0.85	0.95	−0.22	−0.18*	0.00	68.78	0.05*
P4	0.21	0.20	−0.75	0.16*	−0.05*	−20.07	0.06*
P5	−3.14	−2.79	−3.54	0.04	−0.14	−41.75	−0.38

Table 3. Effects of the specific combining ability (SCA) on the agronomic traits of ten crosses.

Crossing	Days to flowering	Days to harvest	Plant height	Fruit diameter	Fruit length	Number of fruits	Fruit weight
1×2	8.76	5.57	0.01	−0.21	0.19	−70.20	−0.55
1×3	−10.05	−8.17	10.22*	0.59*	0.71*	−45.02	0.54
1×4	−5.91	−3.17	10.26*	0.17	0.17	−124.16	−1.33
1×5	−2.30	−2.42	−0.45	0.19	0.28	110.51	1.61*
2×3	−11.55	−7.71	3.51	−0.09	−0.04	−260.91	−1.79
2×4	−8.66	−6.96	1.29	0.42*	0.71*	−4.30	0.30
2×5	−6.55	−6.46	1.58	0.45	−0.14	−56.38	0.19
3×4	5.26	4.53	3.51	−0.43	−0.74	−97.88	−0.29
3×5	−8.38	−5.21	−5.20	0.09	−0.37	118.29*	0.45
4×5	15.01*	15.53*	−2.16	0.29	0.25	116.90*	1.36

combined with recessive alleles of the other parent (Falconer, 1989). In the first case, the cross with highest SCA for the fruit weight variable was the result of crossing two parents with a high GCA (P1 and P3). Meanwhile, the second outstanding cross was the result of crossing a parent with a low GCA and a parent with a high GCA (P5 and P3). These results match the findings of Escorcia *et al.* (2010) and Guerrero *et al.* (2011), who pointed out that a simple cross can obtain a high yield if both parents or at least one of them have high GCA values and high positive SCA effects.

CONCLUSIONS

Information about the yield potential of five *mirador* chili populations was generated. This information can play a fundamental role in the development of genetic improvement programs and for the selection of the method that will be used. The P1, P4, and P3 parents obtained the highest and positive total fruit weight values, with significant differences regarding general combining ability, while the 1×5 and 4×5 crosses recorded the highest values regarding the specific combining ability.

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Presence of flower thrips *Frankliniella schultzei* in ornamental plants

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ABSTRACT
Objective: To report on the presence of *Frankliniella schultzei* in geranium (*Pelargonium hortorum*) and periwinkle (*Catharanthus roseus*) plants in greenhouses in the State of Morelos, Mexico.
Design/Methodology/Approach: We sampled geranium and periwinkle plants in greenhouses in the municipality of Jojutla, Morelos, Mexico. The collected specimens —of light to dark brown color— were processed, mounted on glass slides to be observed through a conventional light microscope, and identified with taxonomic keys.
Results: The specimens collected in the geranium and periwinkle plants presented morphological traits that conform to the species *Frankliniella schultzei*.
Study limitations/Implications: Monitoring the species is necessary because its presence has been identified in at least two regions of Mexico: West and Center.
Findings/Conclusions: *Frankliniella schultzei* had only been located in western Mexico. This report shows that the species is also found in the central region of the country.

Keywords: Morphology, Vector, Pest.

Citation: García-Chávez, M. de los Á., Rodríguez-Arrieta, J. A., Martínez-Fernández, E., López-Martínez, V., Alia-Tejacal, I., Juárez-López, P. & Avonce, N.(2023). Presence of flower thrips *Frankliniella schultzei* in ornamental plants. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i6.2529

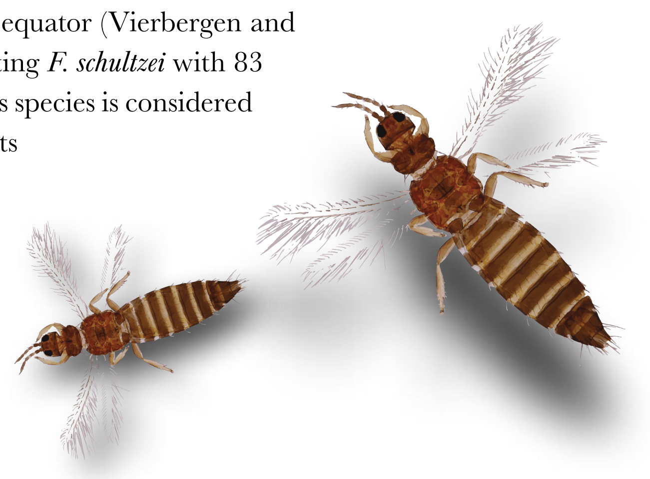
Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 23, 2023.
Accepted: June 26, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 97-101.

INTRODUCTION

Frankliniella schultzei, also known as common flower thrips, is a thrips species with polyphagous habits. Specimens have been morphologically identified in light and dark tones, but their ability to transmit viruses and their geographical distribution are different (Kakkar *et al.*, 2010). Light-colored thrips live mainly north of the equator, while dark-colored ones are found south of the equator (Vierbergen and Mantel, 1991). There are records associating *F. schultzei* with 83 plant species distributed in 35 families; this species is considered a pest of several crops in different parts of the world. Its presence has been reported in vegetables (Feliciano *et al.*, 2008; Kakkar *et al.*, 2012), fruit trees (Carvalho *et al.*, 2020), and ornamentals (Carrizo *et al.*, 2008; Jiménez *et al.*, 2006). In Hawaii, for



example, certain crops are at high risk since *F. schultzei* is a quarantine pest (Yalemar *et al.*, 2001). Studies on the damage and losses caused by this species of thrips show that in melon crops, for instance, the yield dropped by 46% (Diamantino *et al.*, 2021), while in bell pepper crops, the losses amounted to 49% (Da S. Paes *et al.*, 2019).

This species is a notorious vector for the following viruses: Chrysanthemum stem necrosis virus (CNSV), Groundnut bud necrosis virus (GBNV), Groundnut ringspot virus (GRSV), Groundnut yellow spot virus (GYSV), Tomato chlorotic spot virus (TCSV) and Tomato spotted wilt virus (TSWV), as well as Impatiens necrotic spot virus (INSV), all of them belonging to the Tospovirus genus. The dark variant of *F. schultzei* is a competent vector for all these tospoviruses, unlike the light variant, which is not (Reitz *et al.*, 2011). Regarding economic losses due to these viruses, Sevik and Arli-Sokmen (2012) reported that TSWV caused a 42.1% decrease in tomato yield and a 95.5% drop in its commercial value, with estimated losses of around one million dollars in Turkey. In Georgia, USA, losses and economic costs caused by thrips and TSWV in tomato and pepper crops are estimated at over 60% (Reitz, S., and Funderburk, J., 2012).

Likewise, *F. schultzei* has been reported in ornamentals such as *Lathyrus latifolius*, *Tropeaelum majus* (Carrizo *et al.*, 2008), *Chrysanthemum coronarium*, *Mirabilis jalapa*, *Polianthes tuberosa* (Surís and González, 2008), *Hibiscus rosasinensis*, *Vigna caracalla*, *Ipomoea cairica* (Milne and Walter, 2000), *Dimorphotheca ecklonis*, *Lilium* spp., *Jasminum* spp., *Dianthus caryophyllus*, *Dahlia* spp., and *Tagetes erecta* (Jiménez *et al.*, 2006). In Mexico, the first report of *F. schultzei* dates from 2017, with specimens identified in western Mexico: Jalisco and Sinaloa (Johansen-Naime *et al.*, 2017). Until now, there was no other record of this species in the country.

MATERIALS AND METHODS

We conducted two samplings of geranium (*Pelargonium hortorum*) and periwinkle (*Catharanthus roseus*) plants from greenhouses in the municipality of Jojutla, Morelos, Mexico. The samplings took place in November 2020 and June 2021, when the plants were in bloom. To obtain the thrips, mainly from the flowers, we used the tapping technique. The thrips fell on a white sheet, and we collected them with an insect aspirator. We placed the thrips in 70% alcohol until processing them for mounting.

We completed progressive hydration of the thrips in alcohol concentrations of 90%, 80%, 70%, 60%, and 50% for 30 min each. Afterward, they spent two hours in 30% alcohol to be subsequently transferred to 5% NaOH for 20 minutes. With an entomological pin, we perforated their abdomen and massaged to eliminate all intestinal residue. Then they went through progressive dehydration, this time with different timings for each concentration: 50%, 50 min; 60%, 40 min; 70%, 30 min; 80%, 20 min; 90%, 10 min; 100%, 5 min. They were finally left in clove oil for 30 min (Palmer and Mound 1990). For the mounting process, a drop of Canada balsam was added on a slide for each specimen, and the preparations were allowed to dry at 40 °C for one week.

The morphological identification of the specimens was conducted at the Centro de Investigación en Estructuras Microscópicas of the Universidad de Costa Rica (CIEMic-UCR), using the species stored in the Thysanoptera Collection of said institution as reference and employing the taxonomic keys of Mound and Marullo (1996), and Palmer *et al*. (1989).

RESULTS AND DISCUSSION

We obtained a total of 79 specimens, of which we selected 40 for mounting, and taxonomically identified 12, which coincided with the *F. schultzei* species description. All the thrips identified as *F. schultzei* were dark brown females. They had eight-segmented antennae (Figure 1), with metanotal campaniform sensilla absent. The pair of ocellar III setae appeared between the anterior margins of the posterior ocelli, and the comb on tergite VIII was absent (Figure 2).

Figure 1. Dorsal view of *Frankliniella schultzei* (female).

Figure 2. Setae: ioIII: interocular III, poIV: postocular IV, am: anteromarginal, I-III: antennal segments I-III; VII-IX: tergites VII-IX; single arrow: pedicel; double arrow: barely noticeable lateral comb teeth.

According to Vierbergen and Mantel (1991), the tropics and subtropics are the most suitable regions for *F. schultzei*. Therefore, Mexico is a country with an adequate climate for the development of this pest. Although these authors mention that the dark-colored specimens of the species can be found mainly south of the equator, Kakkar *et al.* (2012) have reported dark brown *F. schultzei* in southern Florida.

CONCLUSIONS

Our findings place the common flower thrips *F. schultzei* for the first time in central Mexico and for the second time in the country as a whole. *F. schultzei* is a potential pest for various crops in different states of the Mexican territory. The species would directly affect the crops due to the damage caused by both larvae and adults, as well as their ability to transmit tospoviruses. With this report, we raise alerts to intensify the monitoring of the species and the research on its population and seasonal dynamics.

ACKNOWLEDGEMENTS

We would like to thank the Council of Science and Technology of the State of Morelos (CCyTEM) for funding this work.

We would like to thank CONACYT for granting the first author of this scientific note a scholarship to complete her Ph.D. studies in Agricultural Sciences and Rural Development.

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Colegio de Postgraduados

Comparison of aqueous extracts of *Citrus*×*aurantium* and *Aloe vera* L. as fungistatic control of *Fusarium* spp.

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ABSTRACT

Objective: To evaluate the fungistatic capacity of aqueous extracts obtained from *Citrus*×*aurantium* (sour orange) and *Aloe vera* L. on phytopathogenic fungi of the genus *Fusarium* spp.

Design/Methodology/Approach: Three aqueous extracts were made (aloe peel, aloe peel and gel, and sour orange peel). The inhibition capacity in the in vitro growth of a *Fusarium* spp. strain was measured using the following concentrations: 750 μg/mL, 500 μg/mL, 250 μg/mL, 1 mg/mL, 5 mg/mL, 10 mg/mL, 20 mg/mL, 50 mg/mL, 70 mg/mL, and 100 mg/mL in each extract. The control was sterile distilled water. The radial growth of the mycelium was measured daily, starting from the infected disc up to the edge of its diameter.

Results: The following extracts recorded a good inhibitory response: 100- and 70-mg concentrations of aloe peel and gel in 1 mL of agar; 70-mg concentration of orange in 1 mL of agar; and 100-mg concentration of aloe peel in 1 mL of agar.

Findings/Conclusions: The aqueous extract of aloe had a 47.5% inhibitory potential in the radial growth of *Fusarium*.

Keywords: Aqueous extracts, *Aloe*, *Citrus*, control diseases, *Fusarium*.

Citation: Guerrero-Turriza, H. O., Soto-Barajas, M. C., Rodríguez-Ávila, N. L., Chan-Uc, D. M. (2023). Comparison of aqueous extracts of *Citrus*×*aurantium* and *Aloe vera* L. as fungistatic control of *Fusarium* spp. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i6.2536

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: March 01, 2023.
Accepted: June 28, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 103-110.

INTRODUCTION

Phytopathogenic fungi are a serious problem from the pre-germination until the post-harvest phase, causing great losses and increasing production costs. Consequently, producers look for means to combat the diseases caused by fungi, applying chemical products that prevent the fungal impact on crops. These chemical products cause soil and groundwater pollution and remove biological controllers from the environment. This phenomenon leads to the presence of more pests and diseases, which consequently increases the consumption of chemical agents used to control pests and diseases, increasing production costs.

The fungi of the genus *Fusarium* are highly virulent. In plants, they generate the damping off disease which causes necrosis in the root and stem, preventing the flow of sap which leads to wilting and, ultimately, death.

Image by Obodai26 and Franziska Ingold at Pixabay

The action of plant extracts on pathogens is related to their secondary metabolites (Marcano and Hasegawa, 2002).

The species of the genus *Citrus* are economically important and have multiple benefits for human health; additionally, they contain different types of polyphenols and aromatic steroidal oils of biological importance (Murphy, 1999).

Aloe vera is chemically characterized by the presence of phenolic compounds (Okamura *et al*. 1996). The gel is mainly made up of water, mucilage (and other carbohydrates), organic acids and salts, enzymes, saponins, tannins, amino acids, traces of alkaloids, vitamins, and various minerals (Reynolds, 2004).

The use of inorganic chemical products affects the soil, subsoil, and fauna, causing the loss of biological controllers, which promotes an increase in pests and diseases in crops (Gan and Wickings, 2017). The problem of phytopathogenic fungi is particularly serious, because they are present throughout the production cycle (from pre-germination to post-harvest). The major genera include: *Botrytis*, *Puccinia*, *Rhizoctonia*, *Cladosporium*, *Fusarium*, *Colletotrichum*, *Aspergillus*, *Alternaria*, *Mycosphaerella*, *Hemileia*, *Tilletia*, *Ceratocystis*, *Cochliobolus*, *Scleptium*, *Sclerotinia*, *Erysiphe*, *Sphaerotheca*, *Phytium*, *Plasmopara*, *Peronospora*, and *Phytophtora*. These fungi reduce the quality and quantity of the product (Hosni *et al*., 2013; Castaño-Zapata, 2015).

The organic products obtained from plants that are resistant to the fungi that cause damping off can be used as a control alternative. Their production of metabolites inhibits fungal growth. Some of the advantages of organic products over conventional agrochemical products include a lower impact on biotic and abiotic factors; furthermore, they reduce the impact on the natural controllers of the environment, consequently reducing the presence of pests in crops (Sharma and Malik, 2012; Isman and Grieneisen, 2014; Ordanza-Beneitez, 2017).

The objective of this work is to determine the inhibitory potential of sour orange, and aloe extracts and to characterize the secondary metabolites present in the said extracts.

MATERIALS AND METHODS

The test was carried out from August 2018 to June 2019 at the Laboratorio de Biotecnología Vegetal of the Instituto Tecnológico de Chiná, Campeche. Campeche is located in the southeast of the Mexican territory and has a mostly hot sub-humid climate and summer rains (Aw)1. The average annual precipitation is 1,200 mm and the average annual temperature is 27 °C (max. 36 °C and min. 18 °C).

A large quantity of raw material is required to prepare the extracts. Since not enough material can be obtained from dry aloe, fresh plant material was used, given the high water content of the leaves.

Preparation of the extracts

The aloe leaves were collected from the *ejidos* of Chiná and the sour orange fruits were collected at the Instituto Tecnológico de Chiná, Campeche. The extracts were prepared with hexane, ethanol, and sterile distilled water in a 1:3 ratio (m/v).

Extraction

Aloe extract: peel and gel

The aloe leaves were washed with sterile distilled water and cut in pieces of approximately 3.0 cm^3. The aloe pieces (149.50 g) were placed in a 1,000-mL transparent glass bottle (previously filled with 449 mL of ethanol). The bottle was immediately sealed with cling film (Table 1).

The mixture of macerated plant material was in contact with sterile distilled water for 24 h and was constantly stirred. Afterwards, it was filtered using a funnel and cheesecloth to remove plant material. Holes were made in the lids of the bottles to prevent fermentation; they were stored at 5 °C to be used later in tests for the control of *Fusarium*.

Extraction with a rotary evaporator

Extraction of metabolites from plant extracts

This extraction was carried out using a rotary evaporator (Caframo vv 2000) at the Laboratorio de Agua-Suelo-Planta of the Instituto Tecnológico de Chiná.

The aqueous extracts were purified using a 10-mL syringe and a 0.22-μm MILLEX GV filter. The syringe was used to pour 10 mL of extract through the 0.22-μm wide MILLEX GV filter. The content was collected in a 50-mL test tube and refrigerated at 5 °C. This was carried out with each of the three extracts (sour orange peel; aloe peel, and aloe peel and gel).

Phytochemical analysis of plant extracts

The phytochemical analysis was carried out using the methods described by Valencia-Ortíz (1995), in which the steps that must be followed to identify the families of chemical compounds are explained (Table 2).

Evaluation of the inhibition capacity of the extracts

A one μl of sterile distilled water was placed in an Eppendorf tube. Afterwards, an inoculation loop was used to take a sample of *Fusarium* spp., which was introduced in the Eppendorf tube. The tube was stirred vigorously, to guarantee a homogenous spread of the spores, and previously sterilized 6.0-mm diameter filter paper discs were introduced.

For each test, 250 mL of the PDA culture medium were prepared in a 1:25 ratio (m/v) and sterilized in an autoclave at 116 °C for 30 min.

The culture media with the extracts were prepared in a laminar flow hood (Lumina L-120). First the amount of required extract was added in the Petri dish and subsequently the amount of culture medium was added with the following concentrations: 750 μg/μL,

Table 1. Vegetable extracts of *Aloe vera*, and orange applying a 1:3 ratio (m/v), using water as a solvent.

Extract	Weight of the vegetative material (g)	Volume of dissolvent (mL)	Dissolvent
Aloe: peel	129.90	377.70	Water
Aloe: peel and gel	213	639	Water
Sour orange: peel	201	603	Water

Table 2. Methods and compounds determined by the qualitative chemical analysis of the aqueous extracts (Valencia-Ortiz, 1995).

Method	Compound
Shinoda	Flavonoids
Ferric chloride	Phenols
Gelatin	Tannins
Ninhydrin	Amino group
Lieberman-Burchard.	Triterpenes
Börntraguer.	Quinones
Baljet	Lactones
Dragendorff	Alkaloid
Foam	Saponin
Rosemheim.	Leucoanthocyanidins

500 μg/μL, 250 μg/μL, 1 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml, 50 mg/ml, 70 mg/ml, and 100 mg/ml. Afterwards, the media were stirred and the resulting homogeneous mixture was allowed to solidify. The filter paper discs containing the *Fusarium* spp. strains were placed on the solid mixture of PDA with extracts. For each test, a control was added: an additional 1 μL of PDA culture medium was added to a Petri dish, followed by an infected disc of *Fusarium* spp.

RESULTS AND DISCUSSION

Phytochemical analyses were carried out to identify the chemical compounds found in the plant material used. The plant extracts with ethanol, hexane, and water were tested to determine the inhibitory potential of the various concentrations applied to *Fusarium* spp. strains.

Phytochemical analysis of plant extracts

Table 4 shows the secondary metabolites detected in the qualitative chemical analysis carried out in the plant extracts of aloe and orange.

Therefore, the genera *Citrus* and *Aloe vera* contain various chemical compounds that protect them from external attacks by fungi or insects, since they perform various antifungal and antimicrobial activities.

Table 3. Calculation of the extract-agar PDA dilution of the aloe, and orange extracts for the ethanolic and aqueous tests.

# Annex	Titulo
Annex 1	Calculation of extract-agar PDA dilution for the aqueous extract of aloe vera: peel.
Annex 2	Calculation of extract-agar PDA dilution for the aqueous extract of aloe vera: peel and gel.
Annex 3	Calculation of extract-agar PDA dilution for the aqueous extract of sour orang: peel.

Table 4. Qualitative chemical analysis of the plant extracts of aloe and orange.

Metabolite identified	A:P§	A:PG§	O:P§
Saponin	–	+	–
Steroid saponin	+	–	+
Lactones	++	++	+++
Tannins	–	–	
Leucoanthocyanidins	–	–	+
Xanthones and Flavones	+	–	–
Flavonoids	–	–	++
Steroid triterpenes	–	–	+
Amino group	–	–	+++

Metabolite presence: +++ Highest, ++ Higher, + Present, – Null.
§Vegetative material: Sour orange: peel (O:P.), Aloe: peel (A:P.), Aloe: peel and gel (A:PG.).

Evaluation of the inhibition capacity of the extracts

Aqueous extracts

Fusarium inhibition tests were carried out using extracts made with sterile distilled water and aloe peel, a mixture of pulp and peel and orange peel. Those extracts could easily be dissolved in the PDA culture medium.

Aloe peel

Figure 1 shows the record of *Fusarium* inhibition after five days of observation using sterile distilled water as solvent.

The inhibitory potential of the extracts of *Aloe vera* on the growth of *Fusarium* was minimal; the 100- and 50-mg concentrations obtained the best results. The best result was obtained by adding 100 mg of extract in agar; in the said treatment, the radial growth was 1.70 cm. The second-best result (1.80 cm) was obtained with 50 mg. In those media in which

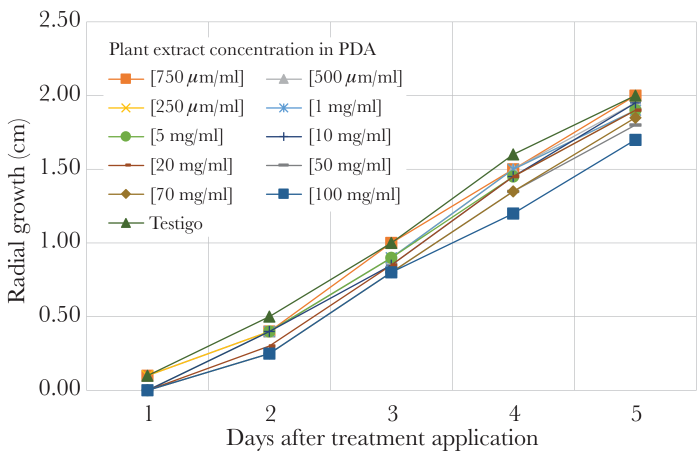

Figure 1. Antifungal activity of the aloe extract (peel) on the radial growth of the mycelium of *Fusarium* spp.

70 mg of extract were added, a growth of 1.85 cm was recorded, while, in concentrations of 20, 5, 1 mg and 250 μg, a radial growth of 1.90 cm was obtained. Conversely, when 10 mg and 500 μg of extract were added, the mycelium reached a 1.95-cm height. Less inhibitory potential was detected in the 700-μg media (2.0 cm); these results are identical to the development of the fungus observed in the control.

Aloe peel and gel

The aloe peel and gel extracts obtained using sterile distilled water as solvent showed a varied behavior on the growth of *Fusarium*. The best results were obtained in the 100-, 70-, and 50-mg concentrations of extract (Figure 2).

The concentration that recorded the best inhibition was 100 mg of extract with a radial growth of 1.05 cm. The 70- and 50-mg concentration did inhibit the development of the fungus to 1.20 cm of radial growth. With the 20-, 10-, 5-, and 1-mg concentrations of extract, the development of the fungus reached 1.80 cm. The 700- and 250-μg concentrations induced a growth of 1.90 cm; this result is similar to the results obtained with the 500-μg concentration (1.95 cm). In the three cases, radial growths were very close to the control (2.0 cm), covering almost the entire petri dish.

Orange

At the end of the inhibition test of the orange extracts on *Fusarium*, a varied radial growth was observed. The concentrations that obtained the best results were 100, 70, and 50 mg of extract (Figure 3).

The concentration that obtained the best inhibition was 100 mg of extract (radial growth: 1.30 cm). The second-best result was obtained in the 70- and 50-mg concentrations, both of which recorded an identical radial growth of 1.50 cm. The 20-, 10-, and 5-mg concentrations obtained a greater radial growth (1.80 cm). For its part, the 1-mg concentration recorded a radial growth of 1.85 cm; this result is similar to the one obtained with 250-μg concentration (1.95 cm). In the 500- and 700-μg concentrations,

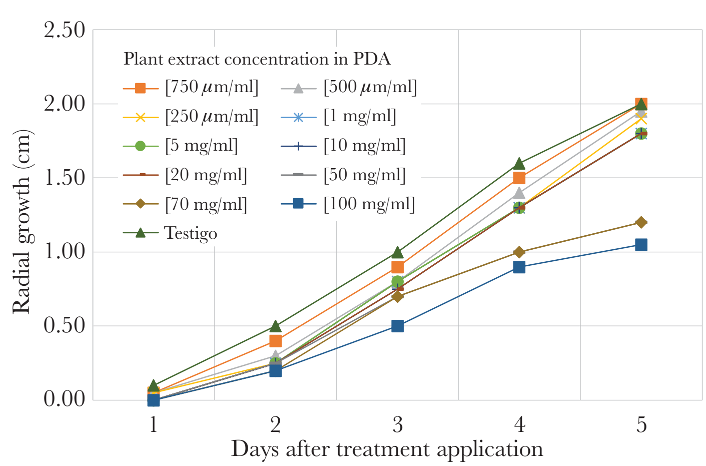

Figure 2. Antifungal activity of the aloe extract (peel and gel) on the radial growth of the mycelium of *Fusarium* spp.

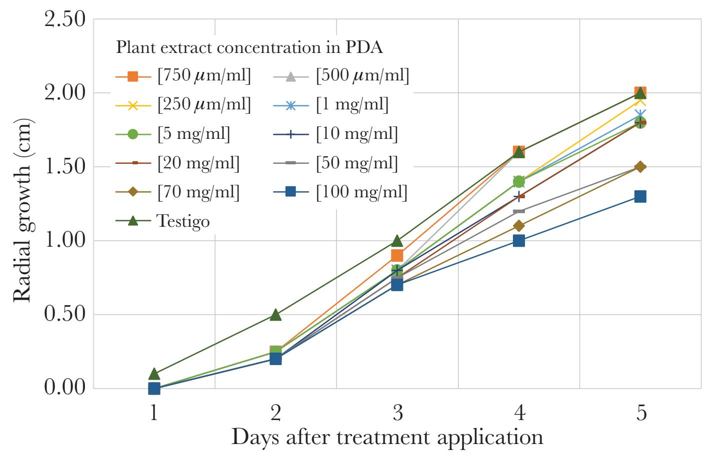

Figure 3. Antifungal activity of the orange extract on the radial growth of the mycelium of *Fusarium* spp.

similar growths (2.00 cm) were obtained, which are identical to those recorded by the control (2.00 cm).

DISCUSSION

The phytochemical analysis of the plant extracts allowed to qualitatively determine the chemical compounds of the peel and gel of the aloe and the peel of oranges. Very high levels were identified in practically all the plant extracts, except for the aqueous extracts of aloe (peel, and peel and gel), which could influence the inhibition of *Fusarium*. In their research, Ruiz and Suarez (2015) mention that the thiol groups of cysteine seem to be the primary targets of sesquiterpene lactones, which causes the inhibition of various cellular functions that lead cells to apoptosis (cell death). In the aloe and orange extracts, the saponin and steroidal saponin chemical compounds could be identified. Ahumada (2016) mentions that the biological properties of saponins include antifungal properties and that their functionality depends on the structural diversity of this metabolite.

Xanthones and flavones were found in the aqueous extract of aloe peel. Reyes *et al* (1997) report that the xanthones were active against *Lenzites trabea*, the fungus responsible for brown rot in wood.

A high presence of flavonols was identified in the orange extracts.

Torres (2017) reports that the *Aloe vera* and moringa extracts affected the development of spore germination (95%) and mycelial growth (68%) of *Colletotrichum* spp., and *Fusarium* spp. Meanwhile, Barkai-Golan (2001) mentions that the evaluated extracts of *Aloe vera* were able to inhibit the spore germination (95%) and mycelial growth (68%) of *Botrytis cinerea*. Finally, Chuchuca (2019) reported that the best alternative to control the crown rot disease caused by fungi in bananas was using 2% *Aloe vera* ethanolic extracts.

CONCLUSIONS

Saponins, lactones, xanthones and flavones were identified in the phytochemical screening of the aqueous extract of aloe peel and gel. These may have an antifungal activity and be responsible for the inhibition of *Fusarium* spp. in the tests carried out.

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Colegio de Postgraduados

Agronomic and phytosanitary characterization of tomato production in the Comiteca-Tojolabal Plateau

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ABSTRACT

Objective: To characterize the agronomic and phytosanitary aspects of the tomato production units of the Comiteca-Tojolabal Plateau, Chiapas, Mexico.

Design/Methodology/Approach: A random sampling of n=76 tomato fields was performed; agronomic practice surveys were applied to their respective farmers, and the phytosanitary status of each site was evaluated.

Results: Tomatoes are grown in shade houses, in plots that mostly do not exceed 0.5 ha. The most common practices are crop rotation, one tomato cycle per year, incorporation of previous crop residues, and fertilization every 5-8 days. For phytosanitary management, foliar sprays are usually performed every 5-8 days. In order to prevent diseases and pests, 67% of producers pre-treat seedlings and 95% apply bactericides and fungicides to the roots during the first 45 days of the crop. The most important pests and diseases are whitefly, thrips, *Bactericera* sp., late blight, virosis, and wilt. The percentage of plants that suffer from root and vascular diseases ranges from 0-38%. However, low values were more frequent. The regional severity of wilting is low, since 90% of the plants evaluated did not present symptoms.

Study Limitations/Implications: This is the first work to describe the agronomic and phytosanitary aspects of tomato cultivation in this region. Additional research with this approach is required and this work will be the basis for further studies.

Findings/Conclusions: Most of the tomato farms have less than 0.5 ha and their farmers carry out intensive phytosanitary management with pesticides. In addition to chemical control, the cultural practices used may contribute to a low incidence and severity of soil-borne diseases. The most important crop diseases are late blight and virosis.

Keywords: pesticides, pest management, agronomic practices.

Citation: Gómez-Domingo, A., Guzmán-Plazola, R. A., Ayala-Escobar, V., & Garrido-Ramírez, E. R. (2023). Agronomic and phytosanitary characterization of tomato production in the Comiteca-Tojolabal Plateau. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2537

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: March 01, 2023.
Accepted: July 25, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 111-120.

INTRODUCTION

Tomato (*Solanum lycopersicum* Mill) is the most important horticultural species in the state of Chiapas, based on the cultivated area, the number of jobs it generates, its production, and its economic benefit (Garrido-Ramírez *et al.*, 2010). In 2021, this state ranked 14th at the national level for its production and 9th for the harvested area (SIAP, 2022a).

Image by Lino Lombardi at Pixabay

The Chiapas tomato becomes relevant in southern-southeastern Mexico, due to its presence in the markets of Tabasco, Campeche, Yucatán, Mexico, Quintana Roo, and other Mexican States, as well as in parts of Central America, which enables its commercial self-sufficiency (Secretaría del Campo, 2014; quoted by InfoRural, 2014). Tomato production in Chiapas is mainly carried out (90%) in Comitán de Domínguez, La Trinitaria, La Independencia, and Las Margaritas, four of the seven municipalities that make up the Comiteca-Tojolabal Plateau Region (CTPR), where more than 1,300 hectares are cultivated using protected systems known as pabellones (SIAP, 2022b). Diseases in the CTPR have caused crop losses and have led some producers to abandon this crop. In addition, this seems to be the reason why yields are lower than in other producing regions in the same state (G. García, 2010). Along with the virosis and late blight problems, tomato wilt is one of the most frequent diseases (Gómez *et al.*, 2014).

The start and development of diseases in cultivated plant populations is the result of a combination of elements such as susceptible host plants, a virulent pathogen, and favorable environmental conditions. However, human intervention must also be taken into consideration, since human activities can contribute or stop the start and development of those diseases (Agrios, 2005).

In order to achieve a better understanding of the factors that influence the disease processes of a crop in a given area, a first approximation is the characterization of the production units in the region of interest. This characterization can help to identify potential relationships between economic, environmental, and social variables and indicators (Vargas and Sánchez, 2015), as well as their effects on biological processes. This measure would facilitate a dynamic interpretation of agroecosystems and the formulation of viable recommendations with a multidimensional approach (Coronel de Renolfi and Ortuño, 2005; Carrillo *et al.*, 2011). Based on the above, the phytosanitary and agronomical characteristics of the tomato production units (TPU) of the CTPR, in the state of Chiapas, were determined given the current lack of published research reports on the subject for this region.

MATERIALS AND METHODS

The research was carried out from 2021 to 2022, in the state of Chiapas. The study area was delimited to the municipalities of La Independencia (16° 15’ 14” N, 92° 01’ 24” W, 1,564 m.a.s.l.) and La Trinitaria (16° 07’ 08” N, 92° 03’ 01” W, 1,553 m.a.s.l.), both of which are part of the CTPR (INEGI, 2017). These two municipalities concentrated 80% of state production in 2021.

A random sampling of n=76 sites was performed. These production units were visited in order to apply surveys to producers and carry out field evaluations.

Information was collected on the following variables: location of the property (town, municipality, and UTM coordinates); cultivated variety; system (crop protection structure); irrigation type; years that tomato has been grown on the farm; crop rotation schedule (crop rotation, continuous management of tomato cycles, or rest period); fertilization (fertilization frequency and nutrients used); soil amendments (any soil amendment incorporated and amendment type); number of tomato crop cycles per year on the same farm; transplant date; and seedling origin (company that provides the seedlings to the producer). Along with general cultivation practices, phytosanitary control with the application of products directed at pest insects and diseases was characterized. In addition, the main phytosanitary problems and their levels of damage were evaluated.

The following variables were evaluated: frequency of applications for pest control and active ingredients used; management of foliar diseases (frequency of fungicide and bactericide foliar sprays and active ingredients used); type of treatment prior to transplantation, in order to prevent disease and pest attack; root disease management (frequency of root product applications and types of products used); record of root and vascular diseases in the two previous cycles; record of foliar diseases in the two previous cycles; incidence of dead plants due to root and vascular diseases (based on a 200-plant sampling); incidence of diseased plants due to root and vascular diseases (based on a 200-plant sampling), including plants killed by wilt and live plants with some level of damage, according to the wilting severity scale of Marlatt *et al.* (1996); wilting severity in the total number of plants sampled in the region (calculating the relative frequency for each severity level, based on the wilting severity scale proposed by Marlatt *et al.* (1996).

RESULTS AND DISCUSSION

Table 1 summarizes the variables evaluated and the values obtained. According to the survey data, the Ponny Express F1 variety is cultivated by 64% of the region's producers, followed by the Serengeti variety (20%). The remaining 16% use other varieties, such as Temible, Gabriela, Ángela, Atrevido, DT-22, and Cantil. Their postharvest quality is the main reason why the Ponny Express F1 and Serengeti varieties predominate in the region, since their firmness and shelf life are better than those of the other varieties. In addition, both varieties have been cultivated in the region for a longer period (13 years), which has resulted in greater knowledge about their requirements and behavior. The rest of the varieties have been introduced in the region in recent years and the particularities of their management are unknown.

A single supplier (Agrocima) supplies seedlings to 57% of the producers in the region; the rest is served by six companies: Pland-Bee (7%), Obed Rodríguez (9%), Plántulas Sta. Rita (9%), Biorquim (9%), Santagro (7%), and Hortamex (3%). Agrocima dominates the seedling market because it was the first to be established and its owners were the pioneers in tomato cultivation in the region. For a long time, it was the only company that supplied seedlings; currently, it has the largest organizational structure and has four branches in the region. Most of the other providers emerged in the last eight years.

Fifty-eight percent of the regional TPUs have less than a quarter of a hectare (ha), while 33% sow 0.25-0.5 ha; 7% have a 0.5-1 ha surface, and only 4% of the producers have farms larger than 1 ha. Nevertheless, none of the TPUs exceeds 2 ha. Tomato production in the CTPR is mainly carried out by small producers. Several reasons determine the presence of these small-scale TPUs, including the availability of farmland, lack of capital for larger-scale investment, lack of irrigation infrastructure, high input costs, and uncertainty in the sale of the harvested product. Most producers grow tomatoes and other important crops (*e.g.*, corn and beans) in small plots. Production costs are high, as a consequence of the

Table 1. Results of the agronomic and phytosanitary evaluation of the tomato production units in the Plateau Comiteca Tojolabal Region, Chiapas†.

Variable	Values (%)
1. Cultivated variety	Serengueti (20 %); Ponny Express F1 (64 %); Others (16 %)
2. Seedling provider	Agrocima (57 %); Pland-Bee (7 %); Obed Rdgz (9 %); Plántulas Sta Rita (9 %); Biorquim (9 %); Santagro (7 %); Hortamex (3 %)
3. Field size	0.1-0.25 ha (58 %); 0.26-0.5 ha (33 %); 0.51-1 ha (6.5 %); >1 ha (2.5 %)
4. Protection structure	Shadow house (96%); Greenhouse (4%)
5. Years of cultivation on the same field	1 (16 %); 2 (34 %); 3 (18 %); 4 (16 %); 5 (8 %); 6 (4 %); 10 (4 %)
6. Crop rotation scheme	Rest (7%); Rotation with corn (92%); Continuous Cycles (1%)
7. Fertilization frequency	Does not fertilize (0%); Every 15 days (8%); Every 10 days (20%); Every 5-8 days (72%)
8. Insecticide application frequency	Occasionally (12%); Every 15 days (5%); Every 10 days (11%); Every 5-8 days (72%)
9. Seedling pretreatment	No treatment (33%); With fungicide (11%); With antibiotic (0%); With fungicide + antibiotic (26%); With fungicide + antibiotic + insecticide (30%)
10. Foliar spray against diseases	Occasionally (4%); Every 15 days (1%); Every 10 days (1%); Every 5-8 days (94%)
11. Application of fungicides-bactericides to the roots	No applications (1%); Occasionally (3%); Every 20 days for 1.5 months (1%); Every 15 days for 1.5 months (45%); Every 10 days for 1.5 months (41%); Every 5-8 days for 1.5 months (9%)
12. Type of soil amendment	None (13%); Corn crop residues (79%); Manure or mature organic matter (8%)
13. Number of tomato crop cycles per year	1 (71%); 2 (21%); 3 (8%)
14. History of root and vascular diseases in the previous two cycles	None (6.5%); Damping off (10.5%): Vascular wilt (0%); Soft stem rot (1%); Symptom complex (82%)
15. Incidence of dead plants on the field*	0 (24%); 1-5 (59%); 6-10 (13%); 11-15 (0%); >15 (4%)
16. Incidence of diseased plants on the field**	0 (9%); 1-10 (55%); 11-20 (29%); 21-30 (4%); >30 (3%)
17. Wilt severity in the population	No symptoms (90%); Mild chlorosis and wilting (2%); Moderate chlorosis and stunting (3%); Severe chlorosis, wilting, and stunting (1%); Dead plant (4%)

†8Results of 76 surveys and evaluations of the crops.
* Dead plants due to root diseases and vascular diseases.
** Diseased plants due to root diseases and vascular diseases.

management of high dependency of crops on agricultural inputs; therefore, producers lack the capacity to establish large surfaces.

Additionally, the commercialization of the product is decisive, given the significant fluctuation in the sale prices; sudden changes in them can lead to a risk of economic losses for the producers. Marketing is controlled mainly by intermediaries, who determine the price of the tomato. Finally, there have been cases of crop losses as a result of pests and diseases, which has led producers to be cautious when they establish a larger cultivation area.

In all cases, the tomato crops are protected with some kind of structure: 96% of them have a shade-house (*i.e.*, a wooden structure with anti-aphid meshes installed); and only 4% have a plastic greenhouse. The CTPR tomato market is mainly regional and the product quality it demands is met with the use of shade-houses. The use of protective covers is essential for tomato cultivation in the region, given the high presence of virus vector pests, including whitefly and thrips. The shade-house system has been more widely adopted than greenhouses, as a result of its lower cost and the fact that no specialized knowledge is required for its installation and management.

A drip irrigation system has been installed in 98.6% of the properties through the use of tapes. This technology is mainly used due to its efficient use of water and its crop fertilization capacity. In terms of time, the crop fields have been sown with tomatoes for 1-10 years, although the crop cycles are not continuous. In 50% of the cases, the producers have been cultivating tomatoes on the same property for 1-2 years, 42% for 3-5 years, and the remaining 8% have used the same property for 6-10 years. Tomato has been used as a crop option in Chiapas since 1960 (Gómez *et al.*, 2015); however, none of the current properties has been cultivated for more than 10 years. The productive actors are dynamic: on the one hand, some choose to abandon tomato cultivation for other activities or to cultivate other species; on the other hand, new producers start growing tomatoes.

Another reason why most of the properties do not exceed 5 years is the constant change to new farmlands. Productivity decreases after a tomato has been grown for a certain number of years in the same site, a phenomenon associated with loss of fertility and an increase in pest and disease populations.

Most of the farmers (92%) practice crop rotation, mainly with corn or with the corn-bean association, 7% let the land rest, and only 1% practice continuous tomato cultivation. Continuous tomato cycles on the same farm lead to a decrease in productivity, as well as an increase in the problems with pests and diseases. Tomato is rotated with corn and beans because these species are important for the producers, since they play a fundamental role in their diet. Additionally, rotation has a positive effect on maintaining productivity and lowering the occurrence of phytosanitary problems.

All producers fertilize the tomato crop: 72% every 5-8 days, 20% every 10 days, and only 8% every 15 days. All producers include sources of macro and microelements in the fertilization program and they also practice soil and foliar fertilization.

Soluble fertilizers (*e.g.*, potassium nitrate, calcium nitrate, potassium sulfate, magnesium sulfate, and magnesium nitrate) are mainly used for this purpose. Other sources are soluble fertilizer complexes that contain both macronutrients and micronutrients, and in some cases, humic, fulvic, and amino acids.

Regarding foliar fertilizers, a wide variety of brands with macro and micronutrient content are used. All the producers fertilize their crops, given its importance in the development of the crop and its proven impact on the yields and the quality of the harvested product. However, this practice is mainly based on the individual and shared experience of the producers and the recommendations of technicians who sell seedlings and agrochemicals in the region. In none of the cases, soil fertility analysis and/or foliar analysis of the crop were carried out. More frequent fertilization has been adopted as a consequence of the clear improvement in crop response to this management. Since crop nutrition is based on soluble fertilizers, a rapid effect has been observed after application; however, this effect does not last long, so frequent applications are required.

All producers apply insecticides: 72% (the majority) at intervals of 5-8 days; 11% every 10 days; 5% every 15 days, and 12% occasionally (as a response to an increase in the pest population). The active ingredients commonly used for pest control are: imidacloprid, thiamethoxam, tiociclam, cyantraniliprole, flonicamid, cypermethrin, abamectin, chlorantraniliprole, spirotetramat, spinetoram, and sulfoxaflor. The major pests are the whitefly, thrips, *Bactericera* sp., and the leaf miner. The control of pests in the crop is fundamentally based on the use of insecticides and anti-aphid mesh. High populations of the main tomato pests (whitefly, thrips, and *Bactericera*) have been observed in the CTPR. Additionally, one of the main limits to the production of tomatoes is the presence of virosis. The association of pests with viral diseases is known in the region. Whitefly and thrips species are widely reported as virus vectors (Fereres *et al.*, 2016). In places located in an area with a cooler climate in the municipality of La Trinitaria where insecticides are applied with less frequency, high populations of these pests are not observed. In TPUs with greenhouse technology, a lower frequency of insecticide was also reported. The use of a wide variety of active ingredients for pest control is a consequence of an increase in the populations of these insects. Additionally, as a result of their constant use, commonly used pesticides have been reported to lose their effectiveness. Therefore, the practice of rotating active ingredients has been adopted to reduce the rapid emergence of insecticide-resistant pest populations.

Seedling treatments prior to transplanting are aimed at protecting them against pests and diseases. Management is variable among producers: 33% do not apply any treatment; 11% immerse them in a solution with fungicide; 26% protect them with a mixture of fungicides plus bactericides; and 30% use a mixture of fungicides, bactericides, and insecticides. Commonly used active ingredients for seedling pretreatment are metalaxyl, propamocarb, fosetyl aluminum, azoxystrobin, gentamicin, streptomycin, and oxytetracycline. Disease problems are common during the seedling stage; therefore, most producers engage in pretreatment. In the cases in which no pretreatment was applied, producers indicated that no soil-borne diseases were observed in previous cycles or that the properties had been only recently used to grow tomatoes. Although root and vascular diseases have been observed in most of farms, the real cause of these symptoms is unknown, and the use of mixtures of products to ensure better control is a common solution.

Foliar applications for disease control take place at intervals of 5-8 days in most cases (93%); the rest apply this treatment every 10-15 days or only occasionally. For foliar applications against diseases, the following products are used: mancozeb, captan, chlorothalonil, copper, cymoxanil, prochloraz, azoxystrobin, propamocarb, dimethomorph, carbendazim, thiabendazole, benomyl, gentamicin, streptomycin, oxytetracycline, and kasugamycin. Late blight is the most important foliar disease, due to its difficult control and aggressiveness. The temperature and humidity conditions favor the infection and development of the pathogen that causes this disease. Other common foliar diseases are early blight and bacterial leaf spots. On the one hand, control products are constantly applied, justified by the losses (washing) caused by the frequent rains in the region and the constant presence of diseases. On the other hand, the control of pests and diseases of the tomato crop is based almost entirely on chemical control; therefore, guaranteeing plant health is highly dependent on the application of chemical products. The use of a wide variety of active ingredients for pest and disease control is also a result of the lack of restrictions on the use of pesticides. As this product is mainly aimed at the national market, producers are not governed by regulations regarding the use of pesticides on tomatoes.

Soil-borne diseases are managed through the direct applications of a mixture of fungicides and bactericides on the roots. These products are applied by 45% of the farmers, at 15-day intervals during a period of 1.5 months; 41% do it every 10 days during the same period; and 9% apply them every 5-8 days. A small percentage (1%) does not apply these products or only does it occasionally (3%). The following products are applied on the roots: metalaxyl, propamocarb, fosetyl aluminum, azoxystrobin, quintozene, thiram, fenamidone, TCMTB, MTC, gentamicin, streptomycin, oxytetracycline, and kasugamycin. The crop is commonly protected against root and vascular diseases during the first half of the crop cycle. If no control treatment is carried out, vascular wilting, damping off, and chlorosis problems are reported during this stage. Applications are less frequent 45 days after transplanting, given the lower incidence of root and stem problems at this stage.

Most tomato producers apply organic amendments to the soil: 79% incorporate residues from the previous crop, 8% apply manure or mature organic matter, and 13% does do not apply amendments. The use of amendments is common, mainly through the incorporation of residues from the previous crop. Most producers have been able to carry out this practice because crop rotation with corn or corn-bean association is a common practice, which does not involve any additional costs. Soil amendments with organic matter, biofertilizer, green manure, or lime to adjust the pH have multiple effects, including an improvement in plant immunity, suppression of pathogenic microorganisms, and the increase of the populations of beneficial microorganisms (Bonanomi *et al.*, 2018; Xue *et al.*, 2019). In addition, they directly provide certain levels of nutrients or the microbial activity altered by this practice modifies their availability (English and Mitchell, 1994).

Seventy-one percent of the producers carry out one crop cycle per year on the same property; 21% carry out two cycles, and only 3% carry out three cycles. Based on their own experience and the recommendations of third parties, one cycle per year has been adopted as a phytosanitary measure, since continuous cycles have been associated with a rapid increase in disease problems and the loss of soil fertility. These results match the findings of Shipton (1979), who indicates that the continuous planting of the same crop can cause the soil to be contaminated with phytopathogenic organisms.

Regarding the root and vascular diseases records, 82% of the producers have observed a complex of symptoms, including damping off, vascular wilting, soft stem rot, chlorosis, and reduced growth; 7% indicated that they had not observed any of those symptoms; 11% reported having observed only the damping off symptom; and only 1% observed soft stem rot. A wide group of pathogens has been identified as the cause of these symptoms, mainly fungi and bacteria found in the soil (Blancard, 2013; Rueda *et al.*, 2014). This suggests that the tomato wilt problem in the region is caused by soil-borne biotic agents.

Regarding foliar diseases, 97% of the producers reported that their crops had been affected by late blight and virosis in previous cycles, considering them as the most important foliar diseases.

According to field evaluations, the incidence of dead plants due to root disease or vascular infection ranged from 0-38%. In 59% of the properties, the incidence ranged from 1-5%. In 24% no incidence of dead plants was reported, and only 4% recorded a >15% incidence. The incidence of plants affected by wilt —which includes dead plants and plants with some level of disease— ranges from 0 to 49%. In 55% of the properties, the incidence of diseased plants ranged from 1 to 10%. In 29%, it ranged from 11 to 20%. At the extremes, 9% did not have wilting symptoms and greater than 30% incidence. The levels of incidence and the severity of wilting are generally low, considering that crop losses have been reported to reach up to 60-70% due to this disease (Ravindra *et al.*, 2015). A low incidence of dead and diseased plants in most of the sites can be explained by the widespread implementation of practices such as crop rotation, amendments, a high frequency of fertilization, one crop cycle per year, and frequent applications of pest and disease control products. As García (2010) mentions, these activities influence root diseases. These practices reduce the amount of inoculum in the soil, improve crop resistance to pathogen attacks, and reduce the number of successful pathogen infections through pesticide applications.

The genetic material is another factor that could be influencing these low disease levels, since the Ponny Express F1 variety has been reported to be resistant to the wilt caused by *Fusarium* sp, (one of the main pathogens associated with tomato wilt). Montiel *et al.* (2020) report the incidence of wilt by *Fusarium* sp. in nine commercial tomato genotypes, with the lowest incidence (0.03%) being reported for the Ponny Express F1 variety. This study recorded incidence levels higher than those reported by these authors for this variety. The occurrence of these differences is natural, since the resistance of crop varieties to pathogens does not remain fixed over time. In addition, under natural field conditions, the presence and interaction with a complex of pathogens and other microorganisms that could alter the resistance levels of the crop is a likely event.

Fernandez-Herrera *et al.* (2013) detected a higher incidence of wilting and a shorter incubation period when jointly inoculating *Phytophthora capsici*, *Rhizoctonia solani*, and *F. oxysporum*, compared to their individual inoculation in tomato plants.

Of the total number of plants sampled in the 76 sites, 90% showed no wilting symptoms, 2% had symptoms of mild chlorosis and wilting, and 3% presented moderate chlorosis and stunt. The rest recorded the highest severity levels: 1% of plants had symptoms of severe chlorosis, wilting, and stunt, and 4% were dead. Based on these data, the severity of the disease in the region is low. The adoption of new crop management practices is likely to have had an influence on these results. In addition to the abovementioned practices that help to reduce the impact of the disease, one of the common practices among producers is the constant change of cultivation land. Usually, after the property has been cultivated for a certain number (4-5 years), it is abandoned as an area for tomato cultivation and is used to plant other crops. The constant change of cultivation areas is a component that can explain a low incidence and severity of wilting in the evaluated properties. Those cultivation practices do not only reduce the amount of inoculum in the soil and give rise to greater physiological resistance in the crop; they also reduce the appearance of populations of more virulent and pesticide-resistant pathogens and decrease successful cases of infection by pathogens.

CONCLUSIONS

The tomato production units in the CTPR are mainly characterized by the use of shade-house structures. Ponny Express F1 is the predominant variety, mainly due to its firmness and long shelf life. The land properties do not exceed 2 ha and most have half a hectare or less. Crop management is intensive, with fertilizers and pesticides applied with great frequency to control pests and diseases. Most producers apply these products on a weekly basis. Practices such as the incorporation of crop residues, crop rotation, and the management of a single tomato cycle per year on the same farm are common among producers.

The level of incidence and severity of tomato wilt in the CTPR is generally low. This could be the result of the agronomic practices carried out by the producers, the variety of tomatoes cultivated, and the characteristics of the pathogens associated with this disease (*e.g.*, the genetic variability that exists in the populations, related to varied levels of virulence within the same species). In addition to tomato wilt, other regionally important diseases are virosis and late blight. Further studies should be carried out to better understand and manage these phytosanitary problems, given the relevance they have gained in the area and the importance of tomatoes as the major vegetable in the state of Chiapas.

ACKNOWLEDGEMENTS

The authors would like to thank the 76 farmers of the CTPR for their active participation during the surveys, as well as the openness and hospitality granted for the phytosanitary evaluation of their tomato crops.

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Colegio de Postgraduados

Aquaculture research with funding from CONAHCYT in three public research centers in Mexico

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ABSTRACT

Objective: Analyze the projects on aquatic species financed by CONAHCYT through the information provided by the Transparency Units (TU) of the public research centers (PRC) CIBNOR, CICESE and CIAD.

Design/Methodology/Approach: The information was requested from the TUs of the centers through the official email of the TUs. The TUs sent the information, and it was organized in spreadsheets for later analysis. For the analysis, only the projects financed by CONAHCYT were considered and the following were selected: the year the project began, the technical manager, the species or species that were the main object of the project, and the amount approved.

Results: The projects financed in the timeline established by each center are shown, as well as the amounts approved, the responsible researchers and the species addressed. Native species are the ones that have obtained the most financed projects, although part of the budget has been applied to invasive alien species. The social incidence is perceptible in some of the financed projects, although it has been a characteristic that has been fairly attended.

Study limitations/Implications: The research has been limited to the information provided by the TUs of PRCs. If more information exists, it is not available through this route.

Findings/Conclusions: The three PRCs propose projects that are mostly on native aquatic species. There has been a decrease in the number of projects funded. The social incidence is barely perceptible in the projects proposed and financed by CONAHCYT.

Key words: Projects, budget, Mexico, social incidence, aquaculture.

Citation: Chong-Carrillo, O., Peña-Almaraz, O. A., Aréchiga-Palomera, M. A., & Vega-Villasante, F. (2023). Aquaculture research with funding from CONAHCYT in three public research centers in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2656

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 14, 2023.
Accepted: June 27, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 121-134.

INTRODUCTION

The System of Public Research Centers (PRC) of the National Council of Humanities, Science and Technology (CONAHCYT for its acronym in Spanish) is a group of 26 Mexican public institutions dedicated to research and higher-level teaching in various

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disciplines of knowledge. The fundamental axes of these centers are research, training of specialized human resources, promotion of scientific progress and generation of technical and scientific information (CONAHCYT, 2023). The centers integrated into this system carry out research of a diverse nature according to the objectives for which they were created, in this way, all of these allow practically all lines of knowledge to be addressed.

Within this system of centers there are three that stand out for attending to research related to aquatic, marine and freshwater organisms, with proven or potential use in cultures for food production: the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE) and the Centro de Investigación en Alimentación y Desarrollo (CIAD).

Even though they are iconic centers in this scientific and technological area, little or nothing is known, in the public sphere, about how their research work has been developed, especially everything related to projects that have been financed by the federal government, through calls issued mainly by CONACYT (now CONAHCYT). The information, which is public, derived from these centers is not easily accessible, nor is it organized in such a way that it can be consulted. Due to the above, the present study, took out the tools that the Mexican State itself has contemplated for access to public information; the National Institute of Transparency, Access to Information and Protection of Personal Data (INAI). This institute has the obligation to provide the public information generated by the agencies and institutions of the federal government (INAI, 2023), which is the case of the CONAHCYT PRCs.

Therefore, the main objective of this study is to analyze certain aspects of the development of research, financed by the federal government, aimed at aquaculture species in PRCs, CIBNOR, CIAD and CICESE, based exclusively on the information provided by the Transparency Units of these centers, through the INAI.

MATERIALS AND METHODS

The Transparency Units (TU) of CIBNOR, CIAD and CICESE, attached to INAI, were requested to obtain the information required to carry out this study, based on the following request sent by email to those responsible:

> *With the intention of developing a postdoctoral study on the impact of public funding on the development of research and culture technology of native and exotic aquatic species, I request the information corresponding to the projects that have been submitted to CONACYT calls and have been approved and funded by the agency. This information is required from the current date to three previous decades, with the amounts approved, the participating researchers and the products obtained. (Sic)*

The three centers sent information through the same means, email, in various formats, spreadsheet files (Excel®), pdf files and images. The information that was not found in spreadsheets was transferred to that format by direct capture. Once the databases were formed in spreadsheets, each center was analyzed.

For the analysis, only the projects financed by CONAHCYT through various calls were considered. From the wealth of information received in each project, the following were selected: the year the project began, the responsible researcher, the species or species that were the main object of the project, and the amount approved. No information was received on the products obtained by the project, so it could not be included as part of the study. With these data, new databases were created that were used for the final analysis.

RESULTS AND DISCUSSION

The information provided by the TUs of each PRC was dissimilar in quantity and quality. CIBNOR was the center that provided the most information on the projects financed by CONAHCYT (92 projects from 1999 to 2022), it also included those that were supported by private companies and other non-federal organizations, but these were not considered in this analysis. CICESE provided information on 27 projects supported between 1993 and 2021 and CIAD 23 between 2011 and 2019. CIBNOR was the public center that provided the most information, in a period of 23 years, while CICESE provided a period of 30 years, and that of CIAD only nine years.

These three centers are not newly created, CICESE and CIBNOR were founded as civil associations in the 1970s and CIAD in the 1980s, although the Mazatlán Unit, the main headquarters for research with aquatic species, was founded in 1993, so it can be considered the youngest of the three (information obtained from the official pages of the aforementioned centers). Subsequently, these centers became part of CONAHCYT's PRCs, however, since their creation they had both state and federal support. Also, almost since its creation, they promoted research related to aquaculture species of commercial interest. Therefore, it is paradoxical that, despite being centers that conduct research and technological development in this sense, they can barely share limited information on the projects financed by the federation through CONAHCYT. The foregoing demonstrates that, although the TUs complied with the obligation to provide the information required by law, these types of requests, even though they refer to their essential and substantive activities, are apparently *sui generis*.

The results by center, based on the analysis provided by their TUs, are presented below. If the information received is partial and does not fully reflect the full scenario (mainly due to the lack of inclusion of projects actually approved, in the periods established by the centers themselves) it is equally important, since it not only draws a panorama that despite being limited, it is real but, in addition, it highlights the capacity of the centers to provide public information. It is necessary to clarify that the projects that only referred to increasing the infrastructure, specific analytical techniques, and laboratory equipment, were not taken into account in all the analyzes since the greatest interest was given to those that were specifically directed to the study of organisms with aquaculture interest.

CIBNOR

Figure 1 shows the number of projects financed by CONAHCYT during the period from 2013 to 2022. Species are included by group, crustaceans, mollusks, fish, microalgae, and those with a social incidence orientation.

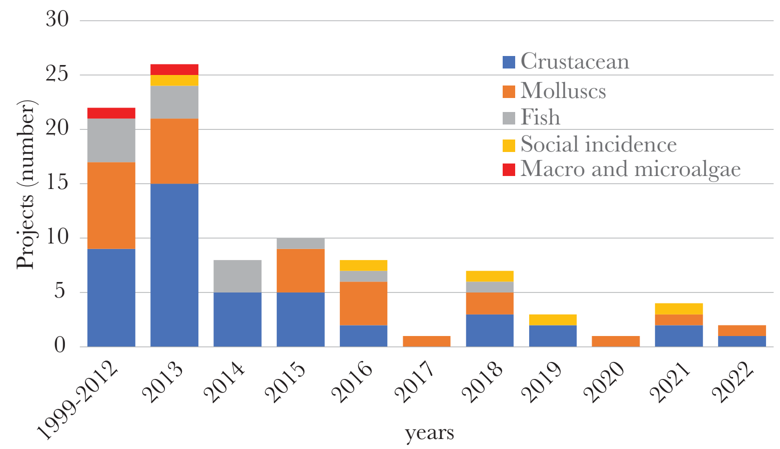

Figure 1. Number of CIBNOR projects financed by CONAHCYT during reported period.

In this Figure it is possible to notice that crustaceans have been the group most widely supported with public financing (44 projects). This group includes species of marine shrimp (mainly *Penaeus vannamei*) and prawns of the genus *Macrobrachium*. The molluscs group is the second most financed with 28 projects. According to the data provided, *Crassotrea gigas* (an exotic species) has received most of the funding, although there are also studies with native species (see table 2). Fish research shows 13 funded projects. Studies financed with macro and microalgae only show one. The social incidence projects total five in that period, which shows that this center began to seek to contribute significantly to this area, even before CONAHCYT's research policies, modified as of 2019, included this aspect in the calls to request funding.

Regarding the amounts approved for the projects, Figure 2 shows that the fish group, despite not being the one with the largest number of projects, is the one that benefited from the greatest financing ($92'540 million in the period), much higher than that of the

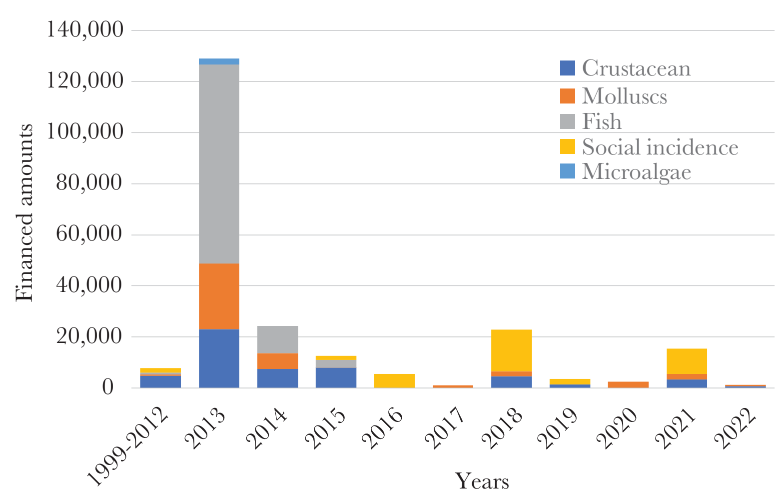

Figure 2. Amounts of CIBNOR projects financed by CONAHCYT during reported period (in thousands of Mexican pesos).

projects financed for crustaceans ($53'484 million) and molluscs ($51'023 million). Of relevant importance, especially at present, is to verify that projects with a social incidence have begun to be reflected in the statistics of the centers. In the case of CIBNOR, these have added almost 37 million in financing, especially since 2018.

Table 1 shows the CIBNOR staff researchers who obtained projects financed by CONAHCYT. For this table, the projects that were directed to the improvement of the infrastructure or acquisition of equipment for laboratories were considered. The ability of some researchers on the CIBNOR staff to obtain federal resources for their studies or for growth in infrastructure and equipment in their specific areas is evident. Of the total number of researchers affiliated to that center, only 45 were supported with funding in the period analyzed.

Regarding the aquatic species studied, Table 2 shows that most of them are native. However, investment has been dedicated to exotic species such as Tilapia (10 projects financed) and the Australian lobster, *Cherax quadricarinatus* (9 projects), both freshwater species that have been considered invasive and currently occupy largely ecological niches. of the country with undetermined consequences. In the same way, the Japanese oyster, *Crassostrea gigas*, has been identified as highly invasive, which has not limited the fact that 15 funded projects are dedicated to its study. Even so, the number of native species that have been subject to budget support is relevant.

CICESE

Despite the fact that the period reported by the CICESE TU covers from 1993 to 2021, the number of projects that received financing from CONAHCYT is much lower than those reported by CIBNOR (Figure 3). The group that showed the most funded projects was fish with 10, while crustaceans and molluscs reported six each; three projects involving microalgae and one addressing bioremediation (not included in the Figure). The projects that were supported for the validation of a product, creation of infrastructure or laboratory equipment, were not included in this Figure or in the one of the approved amounts (Figure 4), because they do not represent the study of a particular organism or organisms.

In relation to the amounts granted to projects, similar to what occurs at CIBNOR, the fish group obtained the most resources ($18,661,000), followed by the molluscs group with a third of that amount ($6' 517,918). The crustacean group only obtained one ninth of what was obtained by fish ($2,090,033) and the microalgae projects were very close ($1,671,900). This clearly reflects the research interests of the different public centers. In the case of CICESE, the projects with the most funding are those aimed at fish and shellfish, despite the fact that the latter only have six projects. Crustaceans are not as important a line of research as they are for CIBNOR. Obviously, the regional vocation of the centers is applied in the study of species with economic interest in their direct area of influence. Table 3 shows the CICESE staff researchers who obtained projects financed by CONAHCYT, in the period from 1993 to 2021. As was the case for CIBNOR, this table did include projects that were aimed at improving infrastructure or acquisition of equipment for laboratories. The difference between CICESE and CIBNOR in this regard is clear. In the case of CICESE, the highest amount obtained by a researcher (10 million) is less than

Table 1. CIBNOR researchers with projects financed by CONAHCYT from 1999 to 2022 (in Mexican pesos).

Researchers	Amounts	Projects
Maeda Martínez Alfonso Nivardo	120,078,000	6
Racotta Dimitrov Ilie Sava	80,208,433	10
Cruz Hernández Pedro	21,297,573	3
Villarreal Colmenares Humberto	20,044,704	5
Ascencio Valle Felipe De Jesús	18,875,402	5
Magallón Barajas Francisco Javier	10,253,001	5
Cortés Jacinto Edilmar	9,633,492	5
Ibarra Humphries Ana María	7,547,495	3
Nolasco Soria Héctor Gerardo	7,421,407	2
Magallón Servín Paola	7,189,041	2
Tovar Ramírez Dariel	6,832,136	4
Mejía Ruiz Claudio Humberto	6,697,106	4
Palacios Mechetnov Elena	6,195,077	6
Espinosa Chaurand Luis Daniel	5,991,000	1
Maldonado García Minerva Concepción	5,883,000	2
Escobedo Fregoso Cristina	4,788,803	2
Pérez Enríquez Ricardo	4,748,461	3
Rojo Arreola Liliana Carolina	3,300,000	1
Hernández Llamas Alfredo	3,236,430	1
Murillo Amador Bernardo	3,194,400	1
Civera Cerecedo Roberto	2,997,812	1
Campos Ramos Rafael	2,950,000	2
Vázquez Juárez Ricardo	2,912,865	3
Mazón Suastegui José Manuel	2,770,492	2
Estrada Muñoz Norma	2,649,800	3
Gómez Anduro Gracia Alicia	2,498,926	2
Peña Rodríguez Alberto	2,471,000	2
López Martínez Juana	1,969,550	2
Gopal Murugan	1,865,000	1
Gutiérrez Jaguey Joaquín	1,676,872	1
Guerrero Tortolero Danitzia Adriana	1,500,000	1
Reyes Alvarado Ana Gisela	1,414,388	1
Mercier Laurence Stephanie	1,396,000	1
Campa Córdova Ángel Isidro	1,391,000	1
Arcos Ortega Guadalupe Fabiola	1,385,706	2
Sánchez Paz José Arturo	1,139,434	1
García Carreño Fernando L.	1,095,436	2
Sicard González María Teresa	1,044,980	1
Valenzuela Quiñonez Fausto	999,321	1
García De Leon Francisco Javier	733,673	2
Martínez Rincón Raúl	609,295	1
Hernandez Lopez Jorge	238,111	1
Monteforte Sánchez Mario	106,000	1
Hernández Cortés Martha P.	100,000	1

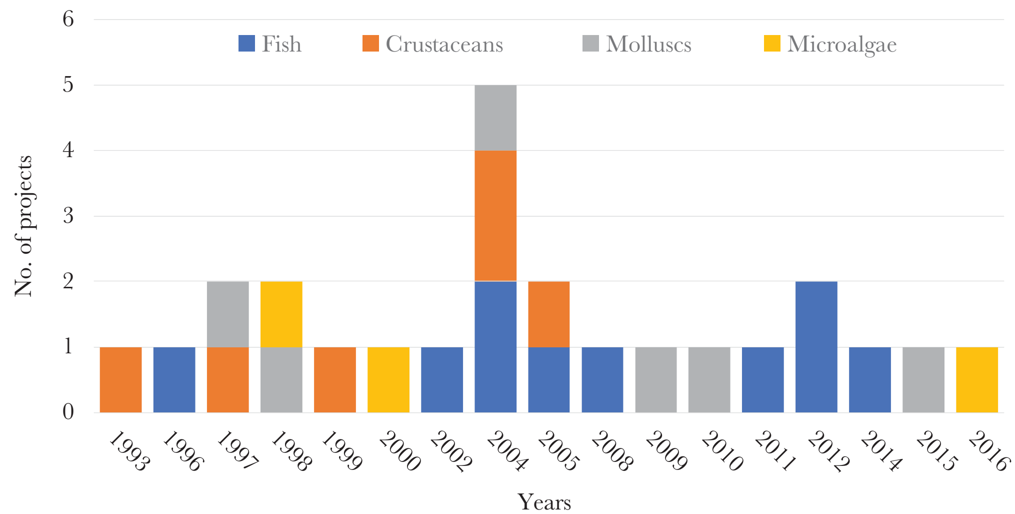

Figure 3. Number of CICESE projects financed by CONAHCYT during reported period.

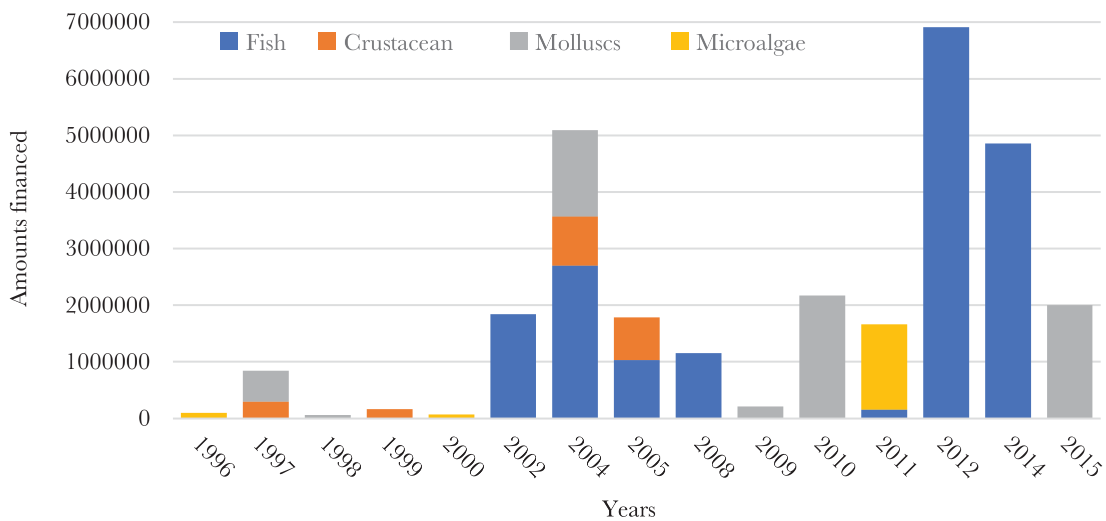

Figure 4. Amounts of CICESE projects financed by CONAHCYT during reported period (in millions of Mexican pesos).

a tenth of that obtained by the most benefited researcher at CIBNOR (120 million). Only 13 CICESE researchers are reported as technical responsibles of financed projects, while at CIBNOR that number is 45. The difference in approval of federal amounts for research between CICESE and CIBNOR is dramatic, especially considering that CIBNOR reports a shorter period than that reported by CICESE. It is also clear that some researchers have the capacity to present financially approved projects, which surely results in benefits for the development of research in their area of expertise or to increase the installed capacity of the laboratories in which they carry out their activities.

The species that have received the most attention for the CICESE projects financed by CONAHCYT are shown in Table 4. Except for Japanese oysters, the Mediterranean

Table 2. Aquatic species subject to study in projects financed by CONAHCYT, at CIBNOR.

Species	Common name	Status
Penaeus vannamei	White shrimp	Native
Crassostrea gigas	Japanese oyster	Exotic
Macrobrachium sp.	Prawn	Native
Oreochromis sp.	Tilapia	Exotic
Cherax quadricarinatus	Australian lobster	Exotic
Seriola rivoliana	Longfin yellowtail	Native
Nodipecten subnodosus	Paw scallop	Native
Mycteroperca rosacea	Leopard grouper	Native
Anadara tuberculosa	Mangrove cockle	Native
Panopea globosa	Geoduck clam	Native
Lutjanus peru	Pacific red snapper	Native
Paroctopus digueti	Pacific pygmy octopus	Native
Crassotrea corteziensis	Cortez oyster	Native
Argopecten ventricosus	Catarina scallop	Native
Megapitaria squalida	Chocolate clam	Native
Crassostrea virginica	American oyster	Native
Haliotis fulgens	Green abalone	Native
Chirostoma estor	Silverside fish	Native

Table 3. CICESE researchers with projects financed by CONAHCYT from 1993 to 2021 (in Mexican pesos).

Researchers	Amounts	No. of projects
Lazo Corvera Juan Pablo	10,046,962	5
Hernández Rodríguez Mónica	6,530,426	4
Sánchez Saavedra M. del Pilar	5,708,454	3
Lafarga de la Cruz Fabiola	5,378,652	2
del Río Portilla Miguel Ángel	2,341,216	2
Barón Sevilla Bemjamín	2,254,300	2
Segovia Quintero Manuel Alberto	1,499,960	1
Bückle Ramirez Luis Fernando	1,169,185	3
Cáceres Martínez Jorge A.	1,050,792	3
Paniagua Chávez Carmen G.	211,900	1
Paniagua Michel José de J.	101,900	1
Ponce Rivas Elizabeth	70,000	1

mussel and tilapia, all the rest are native species. The foregoing demonstrates the interest of this center in research and technological development of species native to the region rather than introduced ones. Financing for the three exotic species mentioned is minimal considering the amounts oriented towards native species. At CICESE, based on the

Table 4. Aquatic species subject to study in projects financed by CONAHCYT, at CICESE.

Species	Common name	Status
Haliotis spp.	Abalone	Native
Oncorhynchus mykiss	Rainbow trout	Native
Crassostrea gigas	Japanese oyster	Exotic
Mytilus galloprovincialis	Mediterranean mussel	Exotic
Panulirus interruptus	California lobster	Native
Paralichthys californicus	California halibut	Native
Oreochromis sp.	Tilapia	Exotic
Penaeus stylirostris	Blue shrimp	Native
Penaeus vannamei	White shrimp	Native
Poecilia sphenops	Molly fish	Native
Procambarus clarkii	Crayfish	Native
Seriola lalandi	Yellowtail kingfish	Native

information provided by the TU, it was not possible to detect any financed project that could be considered as having a social incidence.

CIAD

The period for which information was obtained by the CIAD TU covers the year 2011 to 2019 and is the smallest of the three centers in the present study. The financed projects addressed three main groups: crustaceans, fish, and social incidence. The number of projects for the crustacean group was 12, much higher than for fish with only five, and none for molluscs (Figure 5). It is evident that the line of crustaceans represents great interest for CIAD, which has eleven projects in the period for *Penaeus vannamei*. For fish, *Lutjanus guttatus* (Spotted rose snapper), is the only project financed for a native species of commercial interest, although the social incidence projects (two) have tilapia as a central interest.

Contrary to what is presented in CIBNOR and CICESE, for CIAD the largest amounts of financing are for projects aimed at marine shrimp research ($15,705,328) and in close numbers, for projects with social incidence ($13,133,820). The third and last place in financing is occupied by the fish group with $9,499,064 pesos. It is interesting to note that CIAD, despite having fewer projects financed (compared to CIBNOR and CICESE) has obtained significant financing for its social incidence projects (Figure 6).

Table 5 shows the CIAD researchers who obtained projects financed by CONAHCYT, in the period from 2011 to 2019. Similarly for the other two centers, this table does include projects aimed at improving infrastructure or acquisition of equipment for laboratories. A similar phenomenon occurs in the amounts financed for CIAD projects as the one mentioned for CICESE. There is a striking difference between CIAD and CIBNOR in the amounts obtained by the researchers. Only 14 CIAD researchers are reported as technical managers of financed projects, similar in this sense to CICESE, but well below

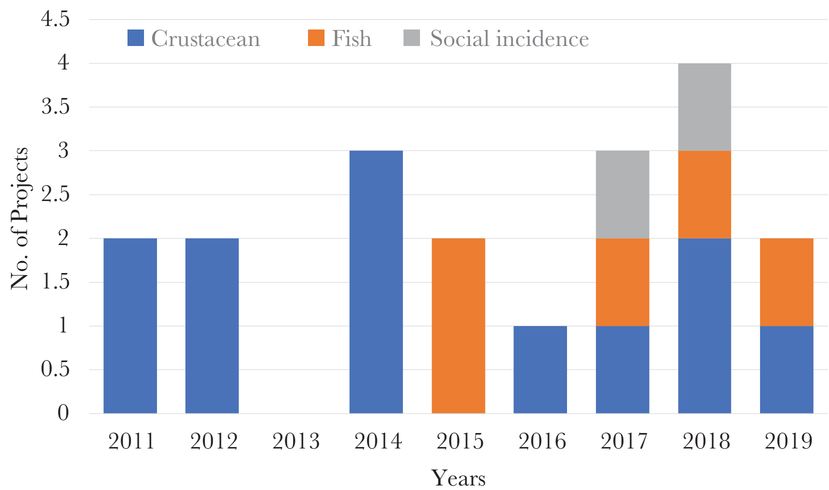

Figure 5. Number of CIAD projects financed by CONAHCYT during reported period.

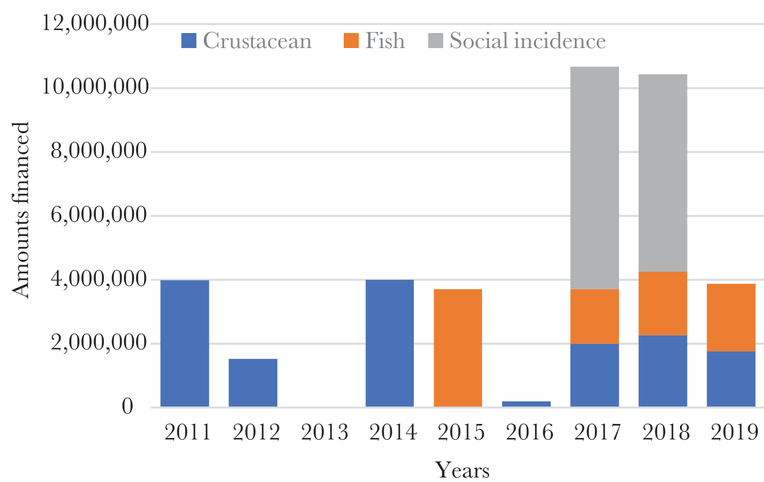

Figure 6. Amounts of CIAD projects financed by CONAHCYT during reported period (in millions of Mexican pesos).

CIBNOR. The difference in the approval of federal amounts for research between CIAD and CIBNOR is dramatic, although the periods are considerably different.

The sum of the projects financed by CONAHCYT to CIAD is a little less than 55 million pesos, while for CIBNOR it is 323 million pesos. However, CICESE only managed to obtain financing for 36 million pesos, over a much longer period. Although only CIBNOR shared information on the projects not financed by CONAHCYT, that is, from external and self-generated resources, and which represent a part of its budget, the other two centers, CICESE and CIAD, must also obtain significant amounts through projects of private companies, specialized services, and consultancies.

In general, several common characteristics can be established among the three centers, regardless of the number of projects reported by the TUs. The most important, from our point of view, is that they spend most of their research on native aquatic species.

Table 5. CIAD researchers with projects financed by CONAHCYT from 2011 to 2019 (in Mexican pesos).

Researchers	Amount	Projects
Martínez Porchas Marcel	8,323,127	3
Javier Ávila Emma Josefina	6,965,820	1
Yépiz Plascencia Gloria	6,255,028	4
Hernández González Crisantema	6,168,000	1
Pacheco Aguilar Ramón	4,885,000	1
Sotelo Mundo Rogerio R.	4,813,500	4
Gasca Silva Silvia A.	3,791,828	1
Chávez Sánchez Ma. Cristina	2,705,000	1
Liera Herrera Raúl A. / Almazán Rueda Pablo	2,187,978	2
Morales Serna Francisco / Soriano Ávalos Anaguiven	2,098,460	1
Soto Rodríguez Sonia	1,767,000	1
Lozano Betancourt Miguel	1,700,000	1
Muhlia Almazán Adriana T.	1,498,000	1
Berlanga Robles César A.	1,476,400	1

The foregoing complies with the concern expressed by the FAO (2010, 2014, 2016, 2020, 2022) in relation to avoiding the introduction of exotic species with potential or known invasive capacity, and the need to research and develop technologies of regional species, native or endemic. Another common characteristic is that, in their staff, there are researchers with a high capacity to present projects that can be financed with federal resources and have been benefited with large amounts. In the reported periods, these researchers have exercised millionaire budgets. Of relevance are the amounts assigned to CIBNOR researchers, in the reported period, much higher than those exercised by the most benefited researchers from the other two centers. In relation to the number of projects financed in the periods reported by the TUs of the three centers, in the case of CIBNOR a dramatic decrease is evident. However, for CICESE and CIAD, this is not perceived. It is worth mentioning that the CIBNOR TU was the one that provided the most information and, consequently, the center where this phenomenon is most clearly perceived. Such a decrease is probably linked to the new regulations that CONAHCYT applies for research financing. The relevance of native species is evident for all three centers, for the majority of the groups (crustaceans, mollusks, and fish) in this study. Among these centers, the crustacean group has been the most extensively addressed through funding provided by CONAHCYT.

Marine shrimps, with *Penaeus vannamei*, as the most important species, are the ones that gather the largest number of financed projects. Other species that have received funding are shrimps of the genus *Macrobrachium* and *Procambarus*, which are also native freshwater species. The first of these is of great socioeconomic importance on both sides of the country as it represents an artisanal fishing resource for riverside communities. The only exotic and invasive species that has received funding is the Australian lobster, *Cherax quadricarinatus*, and it is only present in CIBNOR reports. In the case of this crustacean, native to Australia and New Guinea, its high invasive capacity has been demonstrated in Mexico and other countries (Rodríguez-Cruz *et al.*, 2023); however, it received support from CONAHCYT for various studies, although it is not the only exotic species (classified as invasive) whose projects have been financed. In the case of molluscs, there is a great variety of native species that have been the subject of research and financial support at CIBNOR and CICESE. However, *Crassostrea gigas* is the one that accumulates the most projects, an exotic Asian species and considered invasive in other countries (King *et al.*, 2021). Finally, the group of fish is also addressed in the three centers, with all native species, except for the projects directed at tilapia. Fish of African origin and that has been introduced in a large part of the country as an alternative for fishing and aquaculture (although the ecosystem effects of this introduction have not been properly studied).

Similar to what has happened with terrestrial animal species subject to production, many aquatic species have been introduced into areas outside their natural range of distribution, due to their ease of handling, adequate growth, and survival. Its efficient cultivation allows the generation of food and jobs for the aquaculture sector. Despite the economic and social benefits that, in fact, they provide, their dispersal in native ecosystems has caused disturbances that are of global concern (FAO, 2022). Although most of the native species that have been introduced to Mexico are species whose production is considered, in most cases, of high added value, there are others that were introduced to alleviate the nutritional deficit of rural communities. The greatest exponent of this is the tilapia, *Oreochromis niloticus*, introduced into Mexico in 1964, from the United States and kept at the Temascal Fish Station, Oaxaca (INAPESCA, 2018). The development of the cultivation of this exotic freshwater species has been such that in 2017 almost 150 thousand tons are reported, however, the apparent consumption is almost 276 thousand, so it must be imported from the largest producer in the world, China (Téllez-Castañeda, 2019). Based on the foregoing, we assume, although we cannot confirm it, that both CIBNOR and CIAD addressed the study and cultivation of tilapia and, even more, gave their projects a high impact or social incidence.

In relation to social incidence, the three PRCs declare, on their official web pages, variants of the same manifesto: "*sustainable well-being of Mexican society, especially in its least favored and most vulnerable sectors*" (CIBNOR), "*contributes to generating the knowledge that can contribute to the solution of problems that affect the social and economic environment of Mexico*" (CICESE), "*contribute to sustainable development and the well-being of society*" (CIAD). However, if the projects with a real impact on social welfare (understood as improving the lives of vulnerable sectors) are evaluated, we find that they are few compared to the rest of the majority.

The Special Program for Science, Technology, and Innovation (PECITI) 2021-2024 of CONAHCYT, establishes the strategic bases of a humanities, science, technology and innovation policy that contributes to social well-being, environmental care and the protection of the biocultural wealth of Mexico (CONACHCYT 2023). In these bases, social impact or incidence is considered as one of the fundamental axes for the development of science. Considering the context and the evaluation required by the technology produced in a CONAHCYT PRC, it is also necessary to reflect on whether this technological production has indirect impacts, including social incidence (Aguilar-Navarro, 2023).

In the case of the three PRCs, the research carried out with aquatic species is oriented by simple specific weight in the search for food sovereignty. According to Vázquez-Elorza (2023), one of the fundamental pillars of institutions is the recognition that the results of researchers have high levels of impact, especially in the generation of human resources, the transfer of knowledge and the generation of value in the search for food sovereignty. However, this same author mentions that in order to analyze the phenomenon of food insecurity, both internal and external factors must be taken into account, which makes this analysis complex. Among the factors that he identifies and that are related to this study are two that should be closely related: i) Production: reduced levels of production, productivity and sustainability for national demand, ii) Science and research: lack of projects focused on regional needs to address food insecurity. Aguilar-Navarro (2023) mentions that there are few studies dedicated to accurately determining whether technology developed by the PRCs has had a positive impact on reducing inequality gaps in the different regions of the country. In the PRCs included in this study, there are few projects that are characterized by having social incidence as the common thread. However, apparently it has begun to be an aspect that the centers are beginning to attend to, since the projects detected that involve it are more noticeable in their statistics.

The only project that could be detected in the information provided by the TUs, which includes a native fish in social incidence, is with *Seriola rivoliana*, Longfin yellowtail, at CIBNOR, which demonstrates the interest in transferring its culture technology to population groups traditionally vulnerable. All the remaining projects with evident social incidence, detected in CIBNOR and CIAD, are carried out with an exotic species: tilapia. It is worth asking if these centers will be able, in the immediate future, to increase projects with native aquatic species and with a clear social incidence.

CONCLUSIONS

This investigation reveals that the CIBNOR, CICESE and CIAD TUs do not have organized information that can be transferred to the public in an orderly and simple manner. Based on the information provided, it has been possible to carry out an analysis of the projects directed to the investigation of aquatic species, financed by CONAHCYT. However, this study may not be complete due to the scant information managed by the TUs. All three centers have engaged in significant research with native species through funded projects. This demonstrates the clear interest in developing farming technologies for regional species. However, a good part of the budget granted by CONAHCYT has been for the investigation of exotic species with a proven invasive nature. There is a core of highly prestigious researchers who have been repeatedly benefited, some with very high budgets. The social incidence is barely perceptible in the projects proposed and financed by CONAHCYT and, for the most part, directed to the cultivation of tilapia, an exotic species, rather than native species, with only one registered project.

ACKNOWLEDGEMENTS

The authors wish to thank the Consejo Nacional de Humanidades, Ciencias y Tecnología (CONAHCYT) for the postdoctoral and doctoral fellowships granted to carry out this research. We appreciate the interest shown by Drs. Danitizia Guerrero and Pedro Cruz, from CIBNOR, for directly providing all the information, within their possibilities.

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Colegio de Postgraduados

A Review of the Current Panorama of Glyphosate Resistance among Weeds in Mexico and the Rest of the World

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Citation: Arispe-Vázquez, J. L., Cadena-Zamudio, D. A., Tamayo-Esquer, L. M., Noriega-Cantú, D. H., Toledo-Aguilar, R., Felipe-Victoriano, M., Barrón-Bravo, O. G., Reveles-Hernández, M., Ramírez-Sánchez, S. E., & Espinoza-Ahumada, C. A. (2023). A Review of the Current Panorama of Glyphosate Resistance among Weeds in Mexico and the Rest of the World. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2618

Academic Editors: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

Received: February 19, 2023.
Accepted: June 26, 2023.
Published on-line: August 29, 2023.

Agro Productividad, 16(7). July. 2023. pp: 135-149.

ABSTRACT
Objective: Review current knowledge of weed resistance to glyphosate in the México and the rest of the world.
Design/methodology/approach: The bibliographic review included a systematic study based on various databases, including FAOSTAT, FAO, SIAP and Weed science.
Results: With the introduction of resistant transgenic crops, the use of this herbicide has increased fifteenfold since 1996. Just in the last 26 years, 350 cases of glyphosate resistance among weeds have been reported worldwide; however, multiple resistance has been recorded in 23 weed species in 17 countries around the world.
Limitations on study/implications: The knowledge generated is essential to plan strategies to reduce the use of glyphosate.
Findings/conclusions: In the future, the dependence on this herbicide will result in multiple resistance among weeds, not only in Mexico, but in more parts of the world than those that have been reported to date.

Keywords: Herbicide, glyphosate, resistance, systemic, crops, weed.

INTRODUCTION

Agricultural production in Mexico is based on fruit trees (26.7%), cereals (20.3%), vegetables (18.2%), industrial (13.4%), and fodder (12.4%). The remaining percentage is distributed among dry legumes, ornamentals, oilseeds, tubers, and seeds for sowing. The annual volume of the production amounted to 142.8 million t (SIAP, 2017), but it

Image by M W at Pixabay

increases year after year, partially as a consequence of the use of pesticides —which for years were considered innocuous for human health— to control pests, diseases, and weeds. The word pesticide includes all the chemical products used to control pests and diseases; the most frequently used are: insecticides, fungicides, herbicides, rodenticides, acaricides, and bactericides. Although pesticides have helped to increase the yields of crops, concerns about the environmental risk and their fate have been raised for years (Harner *et al.*, 1999; Tilman *et al.*, 2002; Juraske *et al.*, 2002), partially as a consequence of the secondary effects of these pesticides on the soil and mainly on aquatic microorganisms (Farah *et al.*, 2004).

Weeds compete with crops for sunlight, water, space, and nutrients. Without an adequate control (Figure 1), they can significantly impact harvests (Bayer, 2021); for years, it has been mentioned that herbicides could be used to control weeds (Gruber *et al.*, 2004).

A large number of herbicides are available in the market, and they have different modes of action and agriculture uses; they help to control the weeds present in the crop; however, some farmers still use the plow and animals for weed control (Figure 2).

Figure 1. Corn crop without an adequate weed control, which are larger than native corn plants in Puebla, México.

Figure 2. Farmer using plow and a horse for weed control.

Forming an essential part of the decision-making process involved in weed control. Such is the case of glyphosate, which has become part of the strategies employed to control weeds and to reduce crop yield losses (Danne *et al.*, 2019). More precisely, glyphosate has been part, for approximately 48 years, of the management strategies implemented by farmers in different parts of the world to control weeds. However, the intensive use of this herbicide for weed control in the world's agricultural areas currently needs attention, as a consequence of its toxicity to non-target organisms (Hoodaji *et al.*, 2012).

In terms of its composition, it is a crystalline, white, odorless powder, with a density of 1.704; it is soluble in water and insoluble in organic solvents and has no significant volatilization (Burger and Fernández, 2004). Glyphosate [N-(phosphonomethyl) glycine] is a post-emergent and systemic broad-spectrum herbicide (Jaworski, 1972; Franz *et al.*, 1997). It was developed and first used in the 1970s. At the time, it was considered harmless to human health; that is to say. Frequency of worldwide cases of glyphosate resistance among weeds previously considered non-toxic to animals and humans (Lu, 1995; Rios *et al.*, 2004) and even one of the least toxic pesticides for animals (Franz *et al.*, 1977; Duke *et al.*, 2003; Cerderia *et al.*, 2006). Its proponents even mentioned that, when it was used according to the instructions, it did not create safety problems for human health (Williams *et al.*, 2000).

It should be noted that this herbicide is the substance most frequently used to control weeds in the world (Franz *et al.*, 1997; Woodbur, 2000; Coutinho *et al.*, 2007; Dill *et al.*, 2008; Duke and Powles, 2008; Vila-Aiub *et al.*, 2008) since it was introduced by Monsanto (currently Bayer) in 1974. The first commercial product to include glyphosate was Round up® (Lee and Ngim, 2000), which was used for agricultural, industrial, recreational, forestry, and even domestic purposes (Baylis, 2000; Veiga *et al.*, 2001). However, its residues can be found everywhere, from the soil and the atmosphere to agricultural products, and even groundwater (Songa *et al.*, 2009). Species of crop plants were genetically modified to make them tolerant to glyphosate. This modification allowed their use in the management of weeds during all stages of growth (Duke, 2018). To be more precise, transgenic crops (crops with genetic modification resulting from gene manipulation) were first used in 1996 in Canada, Chile, Australia, China, Brazil, and Mexico (Lee and Ngim, 2000). In 2015, transgenic soybean and cotton crops reached 489 million dollars in profits (Brookes and Barfoot, 2017). However, the most outstanding achievement was the introduction of crops with a bacterial gene that conferred resistance to glyphosate (Dill *et al.*, 2008). As a result, the use of glyphosate in agriculture increased fifteenfold: from 51 million kg (1995) to 747 million kg (2014) (Benbrook, 2016; CIBIOGEM, 2019). However, after a 23-year period, the first case of resistance was reported for *Eleusine indica* (L) Gaertn in Malaysia (Massieu, 2009; Arellano-Aguilar and Rendón, 2017). Currently, weeds are an agronomic, economic, social, and political problem worldwide. Social problems are the indirect results of the effects on the population of high glyphosate applications.

MATERIALS AND METHODS

The bibliographic review included a systematic study based on various databases, including FAOSTAT, FAO, SIAP and Weed science. This review was carried out from

Table 1. Frequency of worldwide cases of glyphosate resistance among weed.

Specie	Year	Country	Specie	Year	Country
Amaranthus hybridus	2013	Argentina	*Conyza sumatrensis*	2009	España
	2014	Argentina		2010	Brasil
	2016	Argentina		2010	Francia
	2018	Brasil		2011	Brasil
Amaranthus palmeri	2005 (2)	Estados Unidos		2012	Grecia
	2006 (3)	Estados Unidos		2016	Francia
	2007	Estados Unidos		2017 (2)	Brasil
	2008 (4)	Estados Unidos		2017	Paraguay
	2009	Estados Unidos		2018	Australia
	2010 (6)	Estados Unidos		2019	Argentina
	2011 (4)	Estados Unidos	*Cynodon hirsutus*	2008	Argentina
	2012 (3)	Estados Unidos	*Digitaria insularis*	2005	Paraguay
	2013 (5)	Estados Unidos		2008	Brasil
	2014 (3)	Estados Unidos		2014	Argentina
	2015	Argentina		2020	Brasil
	2015	Brasil		2020	Paraguay
	2015 (3)	Estados Unidos	*Echinochloa colona*	2007	Australia
	2016	Brasil		2008	Estados Unidos
	2016	México		2008	Venezuela
	2016 (3)	Estados Unidos		2009	Argentina
	2018	Estados Unidos		2009	Australia
	2019	Estados Unidos		2010	Australia
	2020	Estados Unidos	*Echinochloa crus-galli* var. *crus-galli*	2019	Argentina
Amaranthus spinosus	2012	Estados Unidos		2020	Brasil
Amaranthus tuberculatus	2005	Estados Unidos	*Eleusine indica*	1997	Malasia
	2006 (3)	Estados Unidos		2006	Colombia
	2007 (2)	Estados Unidos		2007	Bolivia
	2008	Estados Unidos		2009	Malasia
	2009 (4)	Estados Unidos		2010	China
	2010 (4)	Estados Unidos		2010	Costa Rica
	2011 (3)	Estados Unidos		2010	Estados Unidos
	2012	Estados Unidos		2011	Estados Unidos
	2013	Estados Unidos		2012	Argentina
	2014	Canadá		2012	Indonesia
	2015 (2)	Estados Unidos		2013	Jápon
	2016 (3)	Estados Unidos		2016	Brasil
	2017 (3)	Canadá		2016	Colombia
	2020	Estados Unidos		2016	México
Ambrosia artemisiifolia	2004 (2)	Estados Unidos		2017	Brasil
	2006 (3)	Estados Unidos		2019	Italia
	2007 (4)	Estados Unidos	*Euphorbia heterophylla*	2019	Brasil
	2007	Estados Unidos	*Hedyotis verticillata*	2005	Malasia
	2007	Estados Unidos	*Helianthus annuus*	2015	Estados Unidos
	2007	Estados Unidos	*Hordeum murinum* ssp. *glaucum*	2016	Australia

Table 1. Continues...

Specie	Year	Country	Specie	Year	Country
Ambrosia artemisiifolia	2008 (2)	Estados Unidos	*Hordeum murinum* ssp. *leporinum*	2018	España
	2008	Estados Unidos	*Kochia scoparia*	2007	Estados Unidos
	2010	Estados Unidos		2009	Estados Unidos
	2012	Canadá		2011	Estados Unidos
	2013 (3)	Estados Unidos		2012 (2)	Canadá
	2014	Estados Unidos		2012 (3)	Estados Unidos
	2015	Estados Unidos		2013 (4)	Estados Unidos
	2016 (2)	Estados Unidos		2014	Canadá
	2017	Estados Unidos		2014 (3)	Estados Unidos
Ambrosia trifida	2004	Estados Unidos		2017	Canadá
	2005 (3)	Estados Unidos	*Lactuca saligna*	2017	Australia
	2006 (3)	Estados Unidos	*Lactuca serriola*	2015	Australia
	2007	Estados Unidos	*Leptochloa virgata*	2010	México
	2008	Canadá	*Lolium perenne*	2008	Argentina
	2008	Estados Unidos		2012	Nueva Zelanda
	2009 (2)	Estados Unidos		2013	Portugal
	2010 (2)	Estados Unidos		2015	Nueva Zelanda
	2011	Canadá	*Lolium perenne* ssp. *multiflorum*	2001	Chile
	2011 (2)	Estados Unidos		2002	Chile
Arctotheca calendula	2020	Australia		2003	Brasil
Aster squamatus	2021	México		2004	Estados Unidos
Avena fatua	2018	Australia		2005	Estados Unidos
Avena sterilis ssp. *ludoviciana*	2018	Australia		2006	Chile
Bidens pilosa	2014	México		2006	España
Bidens subalternans	2018	Paraguay		2007	Argentina
Brachiaria eruciformis	2014	Australia		2007	Chile
Brassica rapa	2012	Argentina		2008	Italia
Bromus catharticus	2017	Argentina		2008 (2)	Estados Unidos
Bromus diandrus	2011	Australia		2009	Estados Unidos
Bromus madritensis	2018	España		2010	Argentina
Bromus rubens	2014	Australia		2010	Brasil
Bromus tectorum	2021	Canadá		2010	Estados Unidos
Carduus acanthoides	2019	Argentina		2011	Jápon
Chloris barbata	2018	México		2011	Suiza
Chloris elata	2014	Brasil		2012	Italia
Chloris radiata	2019	Colombia		2012	Nueva Zelanda
Chloris truncata	2010	Australia		2012	Estados Unidos
	2015 (2)	Australia		2014	Estados Unidos
	2015 (2)	Australia		2015	Nueva Zelanda
Conyza bonariensis	2003	Sudáfrica		2015	Estados Unidos
	2004	España		2016	Estados Unidos
	2005	Brasil		2017	Brasil
	2005	Israel		2021	Canadá

Table 1. Continues...

Specie	Year	Country	Specie	Year	Country
	2006	Colombia		1996	Australia
	2007	Estados Unidos		1997	Australia
	2009	Estados Unidos		1998	Estados Unidos
	2010	Australia		1999	Australia
Conyza bonariensis	2010	Grecia		1999	Australia
	2010	Portugal		2001	Sudáfrica
	2011 (2)	Australia		2003	Australia
	2011	Australia		2003	Sudáfrica
	2012	Argentina	*Lolium rigidum*	2005	Francia
	2000	Estados Unidos		2006	España
	2001	Estados Unidos		2007	Israel
	2011	Estados Unidos		2007	Italia
	2002 (5)	Estados Unidos		2008	Australia
	2003 (5)	Estados Unidos		2010	Australia
	2005	Brasil		2013	Australia
	2005 (3)	Estados Unidos		2016	Grecia
	2005	China		2016	España
	2006 (2)	España		2004	Colombia
	2006	España	*Parthenium hysterophorus*	2014	Estados Unidos
	2006	Estados Unidos		2017	México
	2007	República Checa	*Paspalum paniculatum*	2010	Costa Rica
	2007 (3)	Estados Unidos	*Plantago lanceolata*	2003	Sudáfrica
	2009	Estados Unidos	*Raphanus raphanistrum*	2010	Australia
Conyza canadensis	2010	Canadá		2015 (2)	Estados Unidos
	2010	Polonia	*Salsola tragus*	2016	Estados Unidos
	2010 (2)	Estados Unidos		2019	Argentina
	2011	Canadá	*Sonchus oleraceus*	2014	Australia
	2011	Italia		2005	Argentina
	2011	Portugal		2007	Estados Unidos
	2011	Estados Unidos	*Sorgo halepense*	2008	Estados Unidos
	2012	Grecia		2010	Estados Unidos
	2013 (2)	Estados Unidos		2015	Argentina
	2014	Jápon		2018	Australia
	2014	Estados Unidos	*Tridax procumbens*	2016	Australia
	2015	Estados Unidos	*Urochloa panicoides*	2008	Australia
	2016	Hungría		2017	Argentina
	2017	Corea del Sur			
	2019	Francia			

January 2022 to December 2022. The keywords for the search were: weeds, weeds and resistance, resistant weeds, resistance among weeds, glyphosate and weeds, glyphosate, glyphosate resistant weeds, weeds and glyphosate, weeds resistant to glyphosate in Mexico and the world, weeds resistant to glyphosate worldwide.

RESULTS Y DISCUSION

Weed families and damage they cause to crops

In previous years, reports have been published in different parts of the world about the evolution of glyphosate resistance among weeds, based on producer surveys and laboratory, greenhouse, and field experiments (Beckie, 2011). The main weed families that developed the greatest resistance —according to their incidence in several countries, the number of sites of action as they acquired resistance to herbicides, and the number of crops in which they appeared— were: Poaceae, Asteraceae, Amaranthaceae, Brassicaceae, and Chenopodiaceae (Heap, 2011). After 24 years, the families that have developed the greatest resistance to the greatest number of herbicides are: Poaceae, Asteraceae, Brassicaceae, Cyperaceae, and Amaranthaceae (Heap, 2022).

Poaceae is a cosmopolitan family, capable of colonizing all kinds of environments. They are annual or perennial herbaceous plants, with alternate leaves with a linear blade and parallel venation, primary spikelet inflorescence, panicles or racemes, and small and inconspicuous flowers located on the sides of the rachis. They are hermaphrodite and have caryopsis fruits with a single seed. This family has a group of small-sized plants that includes oats (Avena), barley (Hordeum), marram (Ammophilla), and grass (Cynodon) (Soreng *et al.*, 2015; HV, 2022).

The Asteraceae family is represented by chrysanthemums, daisies, dahlias, sunflowers, thistles, chicory, and lettuce. These weeds are distributed all over the world. They are characterized by their capitular reproductive structure, in which the flowers have a sessile arrangement on a widened receptacle (Katinas *et al.*, 2007; Britto and Arana, 2014).

Brassicaceae is a family composed of annual or perennial plants, which have a worldwide distribution, but prefer temperate regions. They include subshrubs (or rarely shrubs), with typically herbaceous stems. Their leaves have a varied morphology (alternate or sometimes opposite). They have hermaphroditic flowers mostly arranged in terminal clusters (rarely in axillary or solitary clusters). Their capsule-type fruits have a highly varied morphology and their seeds have variable color and a smooth or diversely reticulated surface (Monsalve and Cano, 2003).

For its part, the Cyperaceae family has a cosmopolitan distribution: it is frequently found in open areas, ponds, and other humid places (Gómez, 2009). These annual or perennial herbs are hermaphroditic, monoecious or (rarely) dioecious. They have erected or ascending stems (culms), which are often trigonous and solid. They have simple leaves, with sometimes solitary and terminal inflorescence, which is frequently arranged in spikes, clusters, and panicles (among others arrangements), with small, bisexual or unisexual flowers and achenes as fruits (Gómez, 2003).

Finally, the Amaranthaceae family is composed of herbaceous plants, with simple alternate leaves, axillary or terminal inflorescences arranged in clusters or panicles, hermaphroditic or unisexual flowers, and actinomorphic and pixidium fruit, with lenticular, smooth, black, and often shiny seeds (HV, 2022).

The direct damage caused by this weeds diminishes crop yields, as a consequence of the competition for water, nutrients, space, and light. Likewise, their presence increases the investment cost of weeding (labor) and the acquisition of herbicides for their control.

On one hand, producers are penalized when they sell their harvest, as a consequence of the content of weed seeds in the grain and the increase in the moisture percentage. On the other hand, weeds provide refuge for pests and some diseases or they can be alternative hosts; in addition, plots with high weed populations diminish the value of the land (Altieri, 2001; Cordova, 2021). The control strategy will be adjusted to the type of growth of the weeds found on each site, evaluating the presence of broad leaves and narrow leaves and differentiating weeds from crops when there have morphological similarities, such as is the case of *Avena fatua* L. (Poaceae) and wheat regarding species of the *Lolium* and *Phalaris* minor genera, respectively (Malik *et al.*, 2003). Therefore, it is important to determine the morphological characteristics that differentiate these species in their vegetative state (García *et al.*, 2014). Presently, the resistance of weeds to herbicides has become an ecological and evolutionary phenomenon with a broad impact on agricultural production systems (Bonilla *et al.*, 2000; Pinto *et al.*, 2000).

Cases of glyphosate resistance in Mexico and the world

Glyphosate is a widely used systemic herbicide. It belongs to the organophosphate family, although it does not inhibit cholinesterases (Bourgeois *et al.*, 2016). Its mode of action includes several factors, such as: depletion of essential biomolecules synthesized from the shikimic acid pathway, energy reduction in the form of adenosine 59-triphosphate; and carbon diversion in the form of PEP and D-erythrose 4-phosphate, in order to accumulate superfluous shikimic acid and other alicyclic hydroxyacid intermediates of the shikimic acid pathway (Domínguez-Valenzuela *et al.*, 2017). In other words, glyphosate kills plants by suppressing the shikimic metabolic pathway (Diamond and Durkin, 1997). As a consequence of the inappropriate and irresponsible use of glyphosate in agriculture, weed species resistant to this pesticide have been reported in Mexico and all over the world. The countries with the highest number of resistant weed species are: USA (17), Australia (13), and Argentina (9). Worldwide, 45 weed species —including 23 dicotyledons and 21 monocotyledons— are currently resistant to glyphosate, placing it in second place, just behind atrazine (to which 66 species have developed resistance) (Heap, 2022). In just 26 years (1996-2021), 350 cases of glyphosate resistance among weeds have been reported worldwide (Heap, 2022). In Mexico, seven cases of resistance have been reported (Figure 3). Meanwhile, the weed genera with the highest frequency of reports are *Amaranthus*, *Conyza*, and *Lolium* sp.; the United States and Canada stand out with the highest resistance incidents (Figure 4).

Economic importance of weeds

Yield losses to global agriculture caused by weeds range from 30 to 50% (Quintero-Pértuz and Carbonó-DelaHoz, 2016): from 5 to 10% in developed countries, and from 20 to 30% in developing countries (FAO, 2006). In Mexico, yield losses range from 30% (corn and beans) to up to 70% and even 100% (Zita, 2010). For example, during the 2016 agricultural year, the production of corn (a crop affected by 17 species of weeds resistant to glyphosate in the world) (Heap, 2022) amounted to 27,762,480.90 t in Mexico, with a production value of MXN $100,206,306.15 (SIAP, 2017); if we assume that weeds caused 70% losses

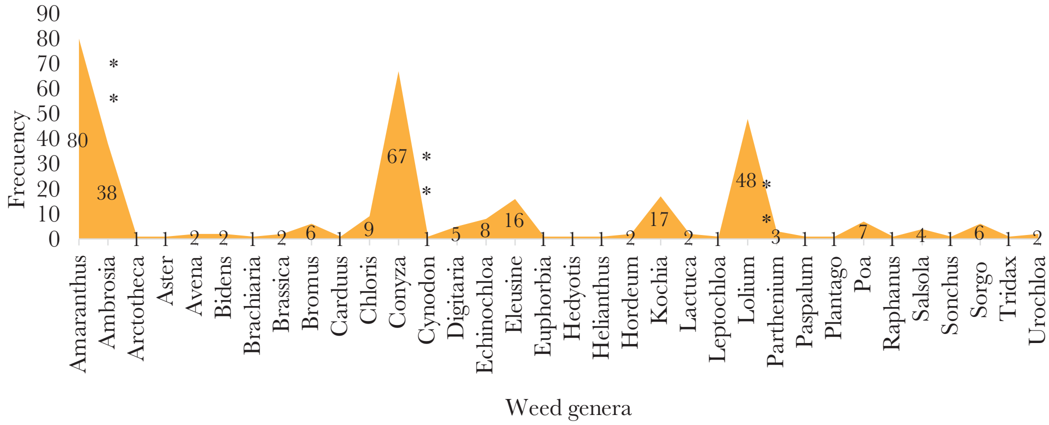

Figure 3. Current perspective of the weed genera based on worldwide reports of glyphosate resistance, **=weed genera with the highest resistance reports.

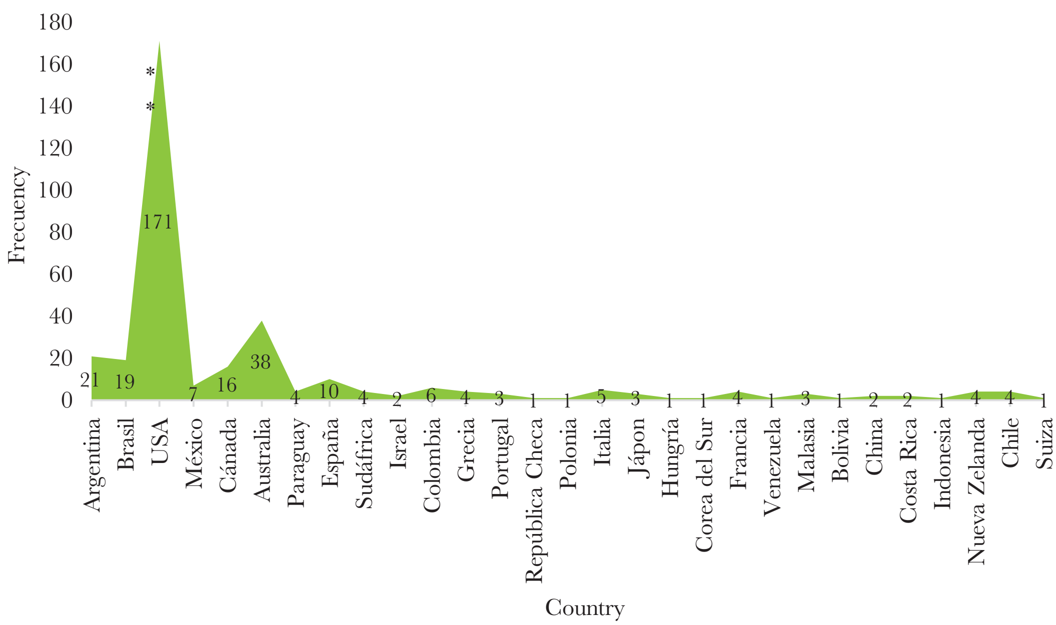

Figure 4. Countries with reports of glyphosate resistant weed species in the world, **= countries with the highest reports of glyphosate resistant.

during the said cycle in Mexico, then the losses amounted to 19,433,736.63 t and MXN $70,144,414.305. In the same year, worldwide production amounted to 1,134,746,667 million t (FAOSTAT, 2022); taking 50% as a reference, the worldwide corn losses caused by weeds during 2016 reached 567,373,333.5 million t. Consequently, in addition to the losses caused by weeds, production costs for the farmers increase, as a result of the increase in the demand for herbicides and practices for their control.

Evolution of herbicide resistant weeds

Herbicide resistance is defined as the evolutionary capacity of a weed population to survive the application of an herbicide that is used to control it (García, 2011). Resistance is attributed to two factors: the frequent use of herbicides in the same place or with the same mode of action, and their propensity to select biotypes of human resources (LeBaron, 1990; Fischer and Valverde, 2000; Beckie *et al.*, 2001); and to an accelerated Darwinian evolutionary process (Vila-Aiub *et al.*, 2008), caused by the selection pressure imposed by the herbicide and genetic mutations (Jasieniuk *et al.*, 1996; FAO, 2022). Herbicides by themselves do not generate mutations in weeds. However, the stress caused by sublethal (low) doses of herbicides could increase the mutation rate (Neve and Powles, 2005; Gressel and Levy, 2006), as has been the case of Australia, where recommended doses are about half the amount used for the same purpose in other parts of the world (Pratley, 1996). However, multiple resistance (in different sites of action) has been recorded in 23 weed species in 17 countries (Table 2) (Heap, 2022) —*i.e.*, in 8.62% of the194 countries recognized by the UN.

Strategies to decrease the use of glyphosate in agriculture

Advances in the search for alternatives to the use of the glyphosate herbicide in Mexico basically comprise three control methods: Cultural, Biological and Chemical. Integrated allow to venture into the agroecological management of weeds in our country. For cultural control, the use of common practices for preparing the land for sowing in production systems that include annual crops is considered, as long as there is a rest period for the land (without sowing: at rest); in which the practices of fallowing, chiseling, tracking, furrow marking and coating are carried out, which spaced at least 20-25 days between them, allows preventing seed production during the rest period and in the case of perennial weeds, expose their underground organs to the sun, achieving their control through dehydration. This practice allows at least 30% control of rhizomes in the case of bindweed *Convolvulus arvensis* L. (Convolvulaceae), which in three consecutive years allows 90% control; although the seed bank remains, which in the case of this species can last at least 20 years.

The black and silver mulches achieve total control (100%) of annual weeds in avocado during the rainy season in Michoacán; which exceeds glyphosate (91%) that requires at least two applications in the season with an approximate cost of MX$2,442.00 against MX$3,500.00 per ha of the cost of the mulch. In walnut, the soil cover mesh controls 100% of annual weeds, dispensing with chemical control with glyphosate; where in the spaces between rows of the crop it is combined with pruning or localized application with bioherbicides, the cost of the soil cover mesh is paid with 1.1 t/ha of conventional walnut or 0.8 t/ha of organic walnut.

The use of cover legumes allows in citrus streets to keep weeds under control, in addition to providing nitrogen, saving water, avoiding erosion, and can be used as fodder or for seed production; Its cost is approximately $7,200.00/ha, compared to the traditional technology at Veracruz that includes four mechanical chaping, as well as three applications of glyphosate with an annual cost of $7,634.00.

Biological control is only available with the use of the morning glory mite *Aceria malherbae* Nuzzaci (Eriophyidae); which is specific for *C. arvensis* and adapts well to the northern regions at Mexico, being considered an alternative to integrate in the management of the species.

Table 2. Multiple resistance in weed species around the world.

Specie	Year	Country	Multiple resistance: sites of action				
A. hybridus	2014	Argentina	c	g			
	2016	Argentina	g	e			
	2018	Brasil	c	C			
A. palmeri	2008	USA	c	c			
	2008	USA	c	c			
	2009	USA	c	c			
	2010	USA	c	c	g		
	2010	USA	c	g			
	2012	USA	c	g			
	2013	USA	c	g			
	2013	USA	c	g			
	2014	USA	c	g			
	2014	USA	c	g			
	2015	USA	c	f	m	g	e
	2015	USA	j	g			
	2016	USA	c	g			
	2016	USA	c	j	g	d	k
	2016	USA	j	g			
	2016	USA	f	g			
	2019	USA	c	g			
	2020	USA	g	e			
A. tuberculatu	2005	USA	c	j	g		
	2007	USA	c	g			
	2007	USA	c	g			
	2009	USA	c	f	j	g	
	2009	USA	c	g			
	2011	USA	c	f	m	g	
	2014	Canadá	c	g			
	2016	USA	j	g			
	2016	USA	c	g	j	g	
	2016	USA	j	g			
	2017	Canadá	c	f	j	g	
	2017	Canadá	c	f	g		
	2017	Canadá	f	g			
	2020	USA	c	f	j	m	g
A. artemisiifolia	2006	USA	c	g			
	2010	USA	c	g			
	2012	Canadá	c	g			
	2015	USA	c	j	g		
	2016	USA	c	j	g		
	2016	USA	c	j	g		
A. trifida	2006	USA	c	g			
	2008	USA	c	g			
	2011	Canadá	c	g			
	2011	USA	c	g			
A. calendula	2020	Argentina	c	i	g		
B. rapa	2012	Argentina	c	g			
C. acanthoides	2019	Argentina	g	e			
C. radiata	2019	Colombia	c	g			
C. bonariensis	2009	USA	l	g			
C. canadensis	2003	USA	c	g			
	2007	USA	c	g			
	2010	USA	l	g			

Table 2. Continues...

Specie	Year	Country	Multiple resistance: sites of action				
C. canadensis	2011	Canadá	c	g			
	2014	USA	c	g			
	2015		l	g			
C. sumatrensis	2011	Brasil	c	g			
	2016	Francia	c	g			
	2017	Brasil	c	l	g		
	2017		f	l	j	g	e
	2017	Paraguay	c	l	g		
D. insularis	2020	Brasil	b	g			
	2020	Paraguay	b	g			
E. colona	2008	Venezuela	b	g			
E. indica	1997	Malasia	b	g			
	2009		b	g	g	h	
	2012	Indonesia	l	g			
	2016	Colombia	l	g			
	2017	Brasil	b	g			
H. verticillata	2005	Malasia	l	g			
K. scoparia	2012	Canadá	c	g			
	2012		c	g			
	2013	USA	c	f	g	e	
	2013		g	e			
	2013		c	g			
	2014	Canadá	c	g			
	2017		c	g	e		
L. perenne	2015	Nueva Zelanda	o	g	h		
L. perenne ssp. *multiflorum*	2002	Chile	c	g			
	2006		b	g			
	2007		b	c	g		
	2008	Italia	b	g			
	2010	Argentina	c	g			
	2010	Brasil	c	g			
	2010	USA	h	g			
	2012	Italia	c	g			
	2015	Nueva Zelanda	o	h	g		
	2015	USA	b	l	g		
	2016		b	c	l	g	
	2017	Brasil	c	g			
L. rigidum	199	Australia	b	c	g	d	
	2003	Sudáfrica	b	l	g		
	2007	Israel	b	c	g		
	2008	Australia	o	g			
	2010		b	c	f	l	g
	2013		l	g			
	2016	España	j	g			
P. annua	2017	Argentina	c	f	g	d	a
R. raphanistrum	2010		c	i	g	e	
S. halepense	2015		b	g			

1=Weed species reported as resistant to glyphosate, 2=Year when the weed was reported as resistant, 3=Location of report, 4=Sites of action as resistant in glyphosate-resistant weeds, a=site of action unknown, b=acetyl CoA carboxylase inhibition, c=acetolactate synthase inhibition, d=microtubule 2 assembly inhibition, e=auxin mimetics, f=PSII-serine 264 Binders inhibitors, g=enolpyruvyl shikimate phosphate synthase inhibition, h=glutamine synthetase inhibition, i=phytoene desaturase inhibitors, j=protoporphyrinogen oxidase inhibition, k=very long-chain fatty acid synthesis inhibitors, l=PS I electron deflection, m=hydroxyphenylpyruvate dioxygenase inhibition, n=inhibition of cellulose synthesis, o=inhibition of lycopene cyclase.

In the case of synthetic herbicides, the use of glufosinate ammonium and paraquat have allowed the control of glyphosate-resistant species such as *Bidens pilosa* L. (Asteraceae), whose control was 35% with glyphosate and 96.5 and 99.8% with alternatives evaluated, with similar or lower costs in the state of Veracruz. In Colima, the lemon crop is kept under control for six months with the application of paraquat + indaziflam; with a cost of MX$1,833.00 against MX$3,342.00 of the six required applications with glyphosate in this period. In addition, in the banana crop, with the application of glufosinate ammonium + indaziflam, costs are reduced by 31%, maintaining control of the annual weed complex for five months, where at least five applications of glyphosate are required.

CONCLUSIONS

In just 26 years, 350 cases of glyphosate-resistant weeds have been reported worldwide, however, multiple resistance has been recorded in 23 weed species in 17 countries around the world. In the future, dependence on this herbicide will result in multiple resistance among weeds, not just in Mexico, but in an increasing number of regions of the world where glyphosate continues to be used irrationally.

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