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APPLICATION OF PHYTOHORMONE ETHREL USING A BRUSH AND BY INJECTION INTO THE TRUNK OF THE SAPODILLA TREE (MANILKARA ZAPOTA (L.) P. ROYEN) TO PROMOTE THE PRODUCTION OF LATEX IN THE NOH BEC COMMON

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Abstract: The production of sapodilla latex has historically had central importance in the economy of families in the rural communities of the center (Mayan zone) and south of the state of Quintana Roo. Production has decreased drastically due to the entry of synthetic rubber for the production of chewing gum. The knowledge accumulated for the extraction of chicle and its processing had been transmitted from generation to generation and is currently being lost due to the risk involved in the activity. The objective of this research is to generate a new method of latex extraction using the phytohormone Ethrel as a natural stimulant, applied with a brush and by injection to the trunk of the tree, preventing the chiclero from climbing the entire trunk of the tree with the risk of a fall. which can leave you disabled or lose your life. The trial was carried out in the Noh Bec ejido, municipality of Felipe Carrillo Puerto in the state of Quintan Roo. The results indicate that there is no significant statistical difference in latex yields between the traditional method and injection application, so producers will be able to have the same latex production by scratching the tree as far as their arm can reach, without climbing the entire tree trunk. tree and risk a fall.

**Keywords:** Tree injection, Mayan jungle, Ancestral knowledge, Forest ejidos, Chewing gum.

# INTRODUCTION

Among the non-timber forest products with the greatest social, economic and environmental importance in the Yucatan Peninsula is sapodilla latex. (*Manilkara zapota*) to produce chewing gum (chewing gum), it was originally produced from this latex. In Quintana Roo, its production has historically had a central importance in the economy of the families of the communities. During the peak of its use, it represented

up to 50% of economic income. According to official statistics, national production of chewing gum in 1980 was 537 tons, declining in 2013 to only 8 tons. The last official record in 2017 was 34 t extracted from Quintana Roo (62%) and Campeche (38%) (SEMARNAT - CONAFOR, 2020). Since the 1950s, there has been a trend to replace natural latex with synthetic products derived from petroleum with a high carbon footprint. Currently, recovery prospects are contemplated due to the growth of natural product markets. There are still some residents in Felipe Carrillo Puerto, Quintan Roo and Calakmul, Campeche who continue to make artisanal chewing gum, although production is low (Por Esto de Quintana Roo, 2021), (Galu Comunicación., 2017).

There are few research works on the extraction of latex from the sapodilla tree. Currently this tree is used for timber purposes, a situation that did not occur before 2007. This can put the stocks of this species at risk, since the use of latex has been greatly reduced due to the impact of changes in the market, price of the product and competition with synthetic chewing gum. The sapodilla tree plays a strategic role in the sustainability of the jungle, since it is one of the main trees that provides food to practically all species of wildlife that participate in the food chain, giving food to herbivorous species that serve as food for carnivores, located at the end of the food chain such as the jaguar and other felines, hence the importance of taking actions so that this species continues to have a greater presence in the Mayan jungles.

The accumulated knowledge for the extraction of chewing gum latex and its processing had been transmitted from generation to generation. Unfortunately, the chicleros who currently have this knowledge have stopped transmitting it to their children, they have decided to send their children to

study so that allows them better employment alternatives. This activity is considered high risk and unprofitable, which is why new generations have preferred to migrate to tourist areas in search of better economic opportunities. In addition to the above, the aging of chicleros who know this activity and their consequent productive inactivity is putting this ancestral knowledge at risk. In an interview with chicleros, they report that, although they consider it important for their children to learn this activity, very few of them know how to do it, since the parents did not worry about teaching them and the children have no interest in continuing practicing it, preferring to go to study. or working in tourist areas.

In search of alternatives to prevent this from happening, a group of professors from `` Universidad Autónoma Chapingo`` and `` Instituto Tecnológico de la Zona Maya`` carried out tests in 2021 and 2022 to generate a new latex extraction method using the phytohormone Ethrel. as a natural stimulant, applied with a brush, as is done in rubber plantations (Hevea brasiliensis Willd.ex A. Juss) and by injection into the trunk of the tree, preventing the chiclero from climbing the entire trunk of the tree with the risk of a fall, which can leave you disabled or lose your life. The trial was carried out in the Noh Bec ejido, municipality of Felipe Carrillo Puerto in the state of Quintan Roo.

The positive results obtained by injecting the trees made it possible to determine the optimal dose that improves yields without putting the tree at risk with high doses that would stress it, defoliate it or risk killing it. The results obtained are presented: optimal doses and the best application method (with a brush or injected).

Tree trunk injection is considered as an alternative method for the application of chemicals with the following advantages: (1)

efficient use of chemicals, (2) reduction of environmental pollution, (3) it is applicable when the methods Traditional methods such as foliar or soil application have been inefficient, ineffective or too difficult to apply (Navarro, Fernandez-Escobar, & Benlloch, (Sanchez-Zamora & Fernandez-1992), Escobar, 2000). (4) each tree can receive an individual dose (Sahagún de la Parra & Sahagún Calderon, 1992). (5) injections can be useful for situations where rapid control is desired without affecting the environment (Tattar, T., Dotson, J., Ruizzo, M. S., & Steward V., 1998). (6) It is considered an ecological measure because the chemicals do not disperse in the air or infiltrate into the soil and water, they are distributed quickly and uniformly to each part of the tree due to its natural transpiration (Takai, Suzuki, & Kawazu, 2003), (Shang, et al., 2014). (7) this technique uses low volumes of products to combat vascular diseases or foliar or internal pests such as; sucking insects, borers or bark beetles since it minimizes product losses due to desiccation, volatilization, washing and photo-degradation (Fernández-Escobar & Benlloch, 1992), (Van Woerkom, et al., 2014), (Wise, 2016), (Hu, Jiang, & Wang, 2017), (Archer, Crane, & Albrecht, 2022). (8) finally, it must be considered that systemic products move through the vascular systems of the plant according to their specific chemicals and know the properties of the product such as; the solubility and the force that the molecules have to dissociate (pKa), organic carbon coefficient and water partition (ml/g or g/g) or carbon adsorption coefficient (Koc) of the active ingredient (A.I.) and the components of a formulation that expresses your level of adherence to the I.A. of carbonrich compounds under a certain environment, such as soil, trunk, leaves or xylem (Doccola, Hascher, Aiken, & Wild, 2012), (Doccola & Wild, Tree injection as an alternative

method of insecticide application., 2012). Understanding this will help predict how it will behave once it is introduced to a tree. In addition to predicting the systematization of chemicals and to which organs they will be delivered, it is important to consider whether there will be a potential danger to pollinators (Van Woerkom, et al., 2014), (Coslor, 2017). Aquino-Bolaños, (Torres-Pérez, García-Trujillo, Cibrian-Tovar, & Méndez-Montiel, 2023) published the manual "Trunk injection: an alternative to nourish, prevent pests or diseases, and promote the production of Latex or resins in trees" where it presents a summary of the main bioassays carried out in Mexico by different authors on trees located in forests and jungles, commercial forest plantations, fruit orchards and in urban areas. The objectives of these bioassays were pest and disease control, tree nutrition in urban and wild areas, and motivation for resin and latex production.

# GOALS

1. Determine the method of extraction of chewing gum latex, using the phytohormone Ethrel, determining the optimal dose for production, without putting the tree at risk, preventing it from becoming stressed or dying.

2. Rescue and avoid the loss of ancestral knowledge of the use of chewing gum latex. Through a more friendly and less risky exploitation method for the producer, helping to transfer this knowledge from generation to generation.

3. Train producers in the new technique for extracting chewing gum latex.

#### METHODOLOGY

The trial focused on analyzing two methods of applying the phytohormone to motivate the segregation of the latex of the sapodilla tree: 1) application with a brush in the scratches of the bark and 2) injection of the phytohormone into the trunk of the tree.

Equipment and materials: 20 ml pipette and syringe, small paintbrush (3 inches), manual drill (branch) or cordless drill (with battery), 24 canvas bags, markers to identify trees (color spray paint) and GPS, Bioinjec-Tree injection kit, phytohormone Ethrel at 21.7% concentration; Adherex brand adherent and distilled water.

#### TREATMENTS

Brush application test: two doses were tested: a) low dose of 2.5% active ingredient (A.I.) and b) high dose of 5% A.I. applied to the scratches that the product made as far as your arm reached. Six diameter categories (CD) were included: 30 – 33 cm; 34 – 36cm; 37 - 40cm; 41 - 43cm; 44 - 46 cm and 47 >= 50 cm. Six witness trees from the same CD to which the phytohormone was applied, applying the traditional method of scratching the trees by climbing to the highest part of the tree and six more trees, one per CD as controls that were only scratched as far as the tree could reach. the producer's arm without applying any product. The total number of trees included in the trial was 24, twelve with Ethrel application and twelve control trees.

The test site was located in the permanent forest area of the Noh Bec ejido, Municipality of Felipe Carrillo Puerto, state of Quintan Roo.



Figure 1. Location of the Noh Bec ejido, Felipe Carrillo Puerto, Quintana Roo (Taken from Tadeo - Noble, et al., 2019)

# STAGES OF THE FIRST TEST APPLYING THE PHYTOHORMONE WITH A BRUSH

First stage: prior to the application of the treatments, with the help of fellow chicleros, the 24 sapodilla trees identified as samagudos with thick bark of the defined CDs were located (georeferenced).

Second stage: The producer scratched, as far as his arm could reach, twelve trees using his traditional machete. Immediately afterwards, the phytohormone was applied with a brush, placing bags at the foot of each tree to collect the latex emitted.

The other twelve witness trees were scratched by the chiclero (six in a traditional way and six only scratched as far as his arm can reach, without applying anything to them).

Stage three: The day after striping the trees, the latex produced was collected, weighing the contents of each bag to control the performance of each tree. Later, the latex was cooked in the traditional way (with fire) to obtain the chewing gum base.

Stage four. The results of the yields per treatment were analyzed using the Statistical Analysis System, SAS see 9.4

Figures 2 and 3 show the process of applying the treatments and the traditional striping of the sapodilla tree.



Figure 2. Scratching the tree to apply Ethrel with a brush and traditional scratching going up the entire trunk of the tree to extract the latex from the sapodilla tree (*Manilkara* zapota)





Figure 3. Scratch and apply Ethrel with a brush and place a canvas bag to harvest the latex.

# RESULTS WITH APPLICATION OF PHYTOHORMONE ETHREL WITH A BRUSH

Figure 4 shows the yields of chewing gum latex in each treatment. The average values of each dose (low and high) and the traditional method are highlighted. The maximum performance in each of the treatments is also indicated. The yield produced in the traditional method in all diameter categories (CD) and its average turned out to be higher than the treatments where the phytohormone Ethrel was applied.

Figure 5 shows the averages of the applied treatments. It is observed that the average obtained in the traditional control is higher than the other treatments where Ethrel was applied. In relation to the yield, the traditional control surpassed the yield between 3 and 2.5 times those obtained with the low dose and the high dose, respectively. The low dose (2.5% A.I.) presented the lowest yields of all treatments.

Figure 6 shows the distribution of yields by treatment. The higher yields stand out with the traditional method of scratching the entire trunk of the tree, compared to the yields generated with the application of the phytohormone with a brush.



Figure 4. Latex performance of treatments, applied with a brush.



Figure 5. Average yields per treatment, brush application



Figure 6. Distribution of yields for the different treatments. T2.5: low dose with 2.5% A.I.; T5.0: high dose with 5.0% A.I.; TRAY: striped only witness; TRAD: traditional witness. Statistical Analysis System SAS 9.4.

Table 1 and Figure 7 show Tukey's standardized rank test for the yields obtained in the applied treatments. The Tukey performance mean test shows that there is a significant statistical difference between the mean latex produced with the traditional treatment and the other treatments, including the application of Ethrel, both at low and high doses. The treatments with low and high doses of Ethrel and the only striped control present similar average latex yields, without a significant statistical difference between them (Figure 7).

Alpha	0.05
Degrees of freedom error	15
Mean square error	0.012885
Critical value of the studentized range	4.07588
Minimum significant difference	0.1889

Table 1. GLM Procedure

Tukey's Studentized Range Test (HSD) for PERFORMANCE

Performance – Tukey Grouping for means of dose (Alfa = 0.05)					
Means covered by the same bar are not significantly different.					
Dose	Estimation				
TTRAD	0.4542				
T5.0	0.1808				
TRAY	0.1608				
T2.5	0.1472				

Figure 7. Tukey test for clustering of latex performance means.

# SECOND TEST APPLYING ETHREL BY INJECTION TO THE TRUNK OF THE CHICOZAPOTE TREE

The diameter categories of the trees were the same as those used in the brush application test. The doses of phytohormone Ethrel (ml of commercial product) were as follows: 1) low: 0.25 ml/cm of normal diameter (DN) of the tree, 2) medium: 0.5 ml/cm of DN of the tree and 3) high: 1 ml/cm of tree DN. The Witnesses were six trees, one per CD, drilled and injected with distilled water, and six control trees applying traditional striping by climbing the entire clean stem of the tree (one/CD category). The total number of trees included in the trial was 30. A minimum of four injection points were applied per tree. The number of trees that were injected was 24 (18 with phytohormone and three with water) and 6 streaked with the traditional method. In total 30 trees included in the trial.

Procedure for injection of the sapodilla tree trunk

**Stage one:** with the help of fellow chicleros, 30 sapodilla trees (Manilkara zapota) identified as samagudos (with thick bark) of the defined CDs were located (georeferenced), supervising that they were in good physical condition and with good phytosanitary condition to include them in the trial, measuring their Diameter at Chest Height (DBH).

Stage two: Once the trees were located, the injection dose was defined based on the DBH and the number of injection points was determined. In smaller diameter trees, three to four injections were applied and in larger diameter trees, up to six injections were applied in order to distribute the application volume. The dosed volume is a function of the dose (low, medium and high) and the centimeters of the Diametric Category (CD). The solution to be injected was prepared according to the appropriate dose. The injection equipment was prepared (pressure calibration so that it does not present air leaks), selecting the most suitable areas at the base of the tree, without physical or biological damage, and proceeding with the drilling of the trunk. A cork stopper was placed in each hole. Subsequently, the injection valves were placed to connect the injection system. To load the correct volume, 20 ml hypodermic syringes were used to deliver the correct dose to each tree. Once the dose was loaded into the device, the pressurization of the equipment (low pressure) began using a Truper brand manual air pump, ensuring that the product was completely introduced into the tree. At the end of this process, you must wait for the system to depressurize before proceeding to disconnect and remove the injection system to wash it and disinfect the materials used.

**Stage three:** The following week the 24 injected trees were striped as far as the producer's arm could reach, harvesting the latex the same day. The six trees (traditional witness) were scratched by the chiclero in a traditional way by climbing the clean trunk of the tree, collecting the latex produced by each tree, weighing the contents of each bag

to control its yield. Later, the latex was cooked in the traditional way (with fire) to obtain the chewing gum base.

**Stage four:** The results of the yields per treatment were analyzed using the Statistical Analysis System, SAS see 9.4



Figure 8. Tree location and inspection, diameter measurement and dose preparation



Figure 9. Phytohormone and injection equipment preparation





Figure 10. Drilling the tree trunk, placing the cork stopper and injection valve





Figure 11. Placement of injection system and correct dose loading at each injection point





Figure 12. Pressurization to introduce the injected dose and depressurization of the injection system to disconnect it and stripe it from the tree trunk.



Figure 13. Traditional striped tree climbing the entire trunk of the tree, latex harvest and cooked gum mark

# RESULTS OF THE APPLICATION WITH INJECTION TO THE TREE TRUNK

The latex yield per tree, treatment and diameter category is presented in Figure 14. The highest average corresponds to the traditional control treatment with 0.394 kg; followed by treatment with the average dose (0.5 ml/cm) with 0.344 kg. It is observed that the highest yield (0.900 kg of latex) was obtained with the average dose in the tree of the largest diameter category (47 >= 50 cm), followed by the tree of the 41 to 43 cm category in the traditional control ( 0.790 kg of latex).

Figure 15 shows the average yields of each treatment (dose) and traditional control. Practically, the absolute difference between the average dose and the traditional control is very small (0.05 kg or 50 g), and in relative terms it represents 12.8% more compared to the traditional control (table 3).

Figure 16 shows the distribution of yields in each treatment and in the two controls. The distribution of treatment yields with medium doses is similar to those obtained with the traditional control.

Table 4 and Figure 17 show Tukey's standardized range test for yields. From this test it is clear that there is no statistical evidence at 95% reliability that there are differences between the application of Etephon and the traditional control, this implies that it makes no difference whether to apply Ethephon in low, medium or high doses (0.25 ml/cm; 0.5ml/cm and high dose 1.0ml/cm) scratching the tree as far as the chiclero's arm can reach, rather than doing it in the traditional way (TTRAD). Although there are no differences between the doses of Etephon, when applying the medium dose better performance was obtained than the other doses (low and high).



Figure 14. Latex yield of treatments with injection to the tree trunk. Where: IBi: Low dose injection, CD i. i goes from 1 to 6; IPROB: Average Low Dose Injection; IMi: Medium dose injection, CD i; i goes from 1 to 6; IPROM: Average Injection Average Dose; IAi: High dose injection, CD i; i goes from 1 to 6; IPROA: Average High Dose Injection; ITti: Traditional witness, CD i; i goes from 1 to 6; PROMITt: Traditional Witness Average; ITmi: Water injection dead witness, CD i; i goes from

1 to 6; promITm: Average Dead Witness.



Figure 15. Comparison of latex performance averages.

Treatment	Average Yield (kg/tree)	Difference/ Traditional (kg/tree)	% Difference/ traditional
Low dose (0.25 ml/cm)	0.248	0.146	-37%
Average dose (0.5 ml/cm)	0.344	0.05	-12.8%
High dose (1.0 ml/cm)	0.295	0.099	-25%
Traditional	0.394	0	0%
Soil scratched	0.113	0.281	-71%

Table 3. Comparison of averages betweentreatments with injection to the tree trunk andthe traditional method.



Figure 16. Distribution of yields for the different treatments. T2.5: low dose; T5.0: medium dose; T10.0 high dose; TAGUA: water injection witness; TTRAD: traditional witness.

Alpha	0.05
Degrees of freedom error	20
Mean square error	42190.42
Critical value of the studentized range	4.23186
Minimum significant difference	354.86

Table 4. Tukey's Studentized Rank Test (HSD) for PERFORMANCE



Figure 17. Tukey test for grouping latex yield means, applying injection to the tree trunk.

## CONCLUSIONS

The results show that there is a statistically significant difference between the yield obtained with the traditional method of scratching the tree by climbing to the top and the treatments where the phytohormone Ethrel was applied with a brush. The best performance was obtained with the traditional method.

It can be concluded that the application of the phytohormone with a brush does not represent a viable alternative to improve the latex extraction method.

The application of Ethrel with a brush

to encourage the extraction of latex from rubber (Hevea brasiliensis), it is a frequent practice with positive results, increasing latex production, howevery the results obtained in sapodilla (Manilkara zopata), they are not encouraging. The anatomical characteristics of Hevea brasiliensis allow the phytohormone to easily penetrate the trunk of the tree when applied with a brush and stimulate the production of latex. In the case of Manilkara zapota, its anatomical structure does not allow the phytohormone to be easily absorbed by the vascular system of the trunk, since, when scratching the tree, it immediately begins to secrete the latex, washing away the phytohormone without allowing it to be absorbed by the tree so that have the desired effect.

The results of the injection application indicate that there is no statistically significant difference in latex yields between the traditional method and the application with Ethrel injection. The best performance with the application of the phytohormone was achieved with the medium dose (0.5 ml/cm of DN), giving a marginal difference with respect to the traditional method of 50 g (table 3). The upper limit of latex yield in the treatment with medium dose was higher (900 gr) compared to the upper limit with the traditional method (790 gr) with a relative difference between both is 12.22% (110 gr) higher in the treatment with medium dose.

Based on the results, it can be concluded that the application of Ethrel to promote the production of chewing gum latex was successful, since producers will be able to have the same latex production by scratching the tree as far as their arm can reach, without climbing the entire tree trunk. tree and risk a fall. It is necessary to make an economic comparison between both methods (traditional and alternative) to see their economic viability.

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