






## Hand-pollination and application of azoxystrobin in the mooring and development of soursop fruit (*Annona muricata* L.) on pruned trees in Las Varas, Nayarit.

## Polinización manual y aplicación de azoxystrobin en el amarre y desarrollo de fruto de guanábana (*Annona muricata* L.) en árboles con poda en Las Varas, Nayarit.

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### ABSTRACT

Soursop (*Annona muricata* L.) holds significant production potential in Nayarit. Soursop production is severely affected by poor pollination and fungal attacks during the flowering period. In this regard, hand-pollination has emerged as an alternative with expectations of enhancing fruit set. This research evaluated the effect of hand- and natural pollination, coupled with fungicide application, on pruned trees in the mooring and development of soursop fruit in Nayarit, Mexico. A total of 236 inflorescences underwent hand-pollination, while 256 were naturally pollinated. Fungicide was applied once to 107 hand-pollinated and 128 naturally pollinated flowers. The results reveal that manual pollination increased the soursop fruit set by 31.64 %. Similarly, the interaction between the fungicide azoxystrobin application and different pruning intensities (100 %, 75 %, and 50 %) increased fruit set by 28.97 %. Finally, the largest fruit sizes, ranging from 34.94 to 60.62 mm in diameter and 59.05 to 103.88 mm in length, were observed with manual pollination and various pruning intensities (100 %, 25 %, and 0 %).

**KEY WORDS :** Fungicide, Pruning, Fruit set

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## RESUMEN

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La guanábana (*Annona muricata* L.) tiene gran potencial de producción en Nayarit. La producción de la guanábana se ve afectada severamente por mala polinización y ataque de hongos en periodo de floración. Al respecto, la polinización manual es una alternativa que ha generado expectativas en el incremento del cuajado de los frutos. En esta investigación se evaluó el efecto de la polinización manual y natural con la aplicación de fungicida en árboles con poda en el amarre y desarrollo de guanábana en Nayarit, México. Se polinizaron y etiquetaron 236 inflorescencias de manera manual y 256 polinizadas de manera natural. Con atomizador se aplicó en una ocasión fungicida a 107 flores polinizadas de manera manual y 128 de manera natural. Se encontró que la polinización manual incrementa 31.64 % el amarre de frutos de guanábana, de igual manera, la interacción entre la aplicación del fungicida azoxystrobin y diferente intensidad de poda (100, 75 y 50 %) incrementó en 28.97 % el amarre de frutos. Finalmente, el mayor tamaño del fruto con medias entre 34.94 a 60.62 mm de diámetro y 59.05 a 103.88 mm de longitud se evidenció con la polinización manual y diferente intensidad de poda (100, 25 y 0 %).

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**PALABRAS CLAVE:** Fungicida, Poda, Cuajado de frutos

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### Introduction

Soursop (*Annona muricata* L.) is a vital alternative fruit crop in Mexico, contributing 30,790.70 t per year, with 75 % (23,230.08 t) concentrated in Nayarit. Key producing areas in Nayarit include Compostela (2,371 ha), San Blas (52.40 ha), and Tepic (12 ha), with yields of 10.63, 8.86, and 8.29 t ha<sup>-1</sup>, respectively (SIAP, 2022). Soursop production in Nayarit faces challenges such as limited irrigation technology, pest and disease management, manual pollination techniques, and pruning, resulting in yields of 10.53 t ha<sup>-1</sup>, comparable to Veracruz (10.55 t ha<sup>-1</sup>) and Colima (10.06 t ha<sup>-1</sup>) (SIAP, 2022).

Soursop is significantly impacted by poor flower pollination. In most orchards, the yields obtained are incompatible with the total number of flowers produced by the plant, due to the late natural pollination process inducing early flower drop or drying (Rebolledo *et al.*, 2009). The low fruit set is mainly due to the behavior of floral dichogamy in soursop, that is, hermaphrodite flowers that have female and male parts with outdated maturation, causing that there is no self-pollination (Pinto de Lemos, 2011; Rebolledo *et al.*, 2009). Additionally, the closed preantesis and early anthesis floral morphology hinder wind and insect pollination, with 10 % of the flowers being self-fertilized and lacking nectar (Peña *et al.*, 2002; Franco-Mora *et al.*, 2001).

Rebolledo *et al.* (2009) reported that environmental conditions significantly influence natural pollination, with optimal conditions requiring 80 % relative humidity and a temperature of 22 °C. Temperatures exceeding 28 °C and relative humidity below 60 % result in flower and fruitlet drop, causing up to 80 % of deformed fruits, further impacting production due to quality and size (Cambero-Ayón *et al.*, 2019; Betancourt-Aranguré *et al.*, 2019) In arid locations, rapid stigmatic liquid drying prevents pollen germination, leading to stigmas falling and blocking the sexual process, impeding ovarian fertilization (Rebolledo *et al.*, 2009; Cárdenas-Torres, 2002).

Given this context, hand-pollination has emerged as a strategy to enhance fruit set. In countries like Brazil, this technique is established among small producers, increasing tree production and fruit quality by up to 50 % (Oliveira *et al.*, 2005). Hand-pollination practices should be done in flowers that are on thick branches that support the fruit load and should take advantage of the characteristics of this fruit species that can generate flowering in lateral buds of the whole plant, so that pruning is necessary to design the required tree canopy. In Mexico, high-density orchards have demonstrated that pruning contributes to yields of up to 12.79 kg per tree and 28.42 t ha<sup>-1</sup> (Reyes *et al.*, 2018). Recent studies indicate that fungicides such as maxtrobyn and flutriafol can aid in controlling anthracnose development on soursop inflorescences and leaves (Betancourt-Aranguré *et al.*, 2019; Hernández-Guervara & López-Rodríguez, 2019).

Given the challenges in yield and fruit malformation faced by soursop, evaluating the effect of hand- and natural pollination with fungicide application in pruned trees is imperative for soursop cultivation in Nayarit, Mexico.

## Material and Methods

The study was conducted in a 4-hectare commercial rainfed soursop orchard, five years old, situated in the ejido El Capomo, Las Varas (21°7'39" N and 105°10'6" W) in the Municipality of Compostela (Nayarit), at an altitude of 80 meters. Weeding was performed with a machete before selecting the experimental plot. The leaves were isolated in the drip zone of each tree and fertilized by broadcasting. In July 2020, at the beginning of the rainy season, a compound mixture (ammonium sulfate, DAP, potassium sulfate, boron, magnesium sulfate, manganese sulfate, and zinc sulfate) was applied at a rate of 1 kg per tree. A second fertilization took place in September 2020 with 300 g per tree of the original Terratec mixture (urea, man, DAP, sam, phosphonitrate, KCl, sop, sulfomag).

During the cleaning process and prior to the flowering of the soursop plants, applications of insecticides, including copper oxychloride (2.5 mL L<sup>-1</sup>), permethrin (0.36 mL L<sup>-1</sup>), and imidacloprid (1 mL L<sup>-1</sup>), were made.

### Hand-pollination

A total of 236 inflorescences, mature or receptive at stage 4 and displaying a sulfur yellow color (Escobar *et al.*, 1986), were randomly selected in June 2021. These were distributed among

trees with different pruning percentages (100 %, 75 %, 50 %, 25 %, 0 %). The inflorescences were hand-pollinated using pollen obtained from mature inflorescences collected manually and stored in #5 paper bags one day prior, kept at room temperature under shade. The collected pollen was stored in a glass jar and applied with a fine bristle brush (#10) using a gentle cross-shaped movement on the gynoecium of the inflorescence. Pollination was conducted between 9-and 12 a.m., coinciding with the receptivity of the female organs. Priority was given to inflorescences located on primary branches and carrying capacity. After pollination, the fungicide Maxtrobyn (Azoxystrobin) was applied to 107 inflorescences using an atomizer on a single occasion at a dose of 3 mL L<sup>-1</sup>, while the remaining 129 inflorescences did not receive the fungicide. All inflorescences were recorded and labeled for follow-up.

### **Natural pollination**

A total of 256 inflorescences, mature or receptive at stage 4 and displaying a sulfur yellow color (Escobar et al., 1986), were randomly selected in June 2021. These were distributed among trees with different pruning percentages (100 %, 75 %, 50 %, 25 %, 0 %). They were not naturally pollinated. The fungicide Maxtrobyn (Azoxystrobin) was applied to 128 inflorescences with an atomizer on a single occasion at a dose of 3 mL L<sup>-1</sup>, while the remaining 128 inflorescences did not receive the fungicide. All inflorescences were recorded and labeled for follow-up.

### **Percentage of mooring**

Mooring percentage was considered from the moment the first morphological change of the flower was observed, transitioning from the brown fruit bud to a brown-spiked ball, indicating the onset of fruit growth.

### **Fruit development (diameter and length)**

Using a Vernier caliper, the polar and equatorial diameter of fruits during their tree-bound development was measured. The initial measurement was taken at the first morphological change in the flower, from the brown fruit bud to a brown-spiked ball. Subsequent measurements were taken at 15-day intervals.

### **Statistical analysis**

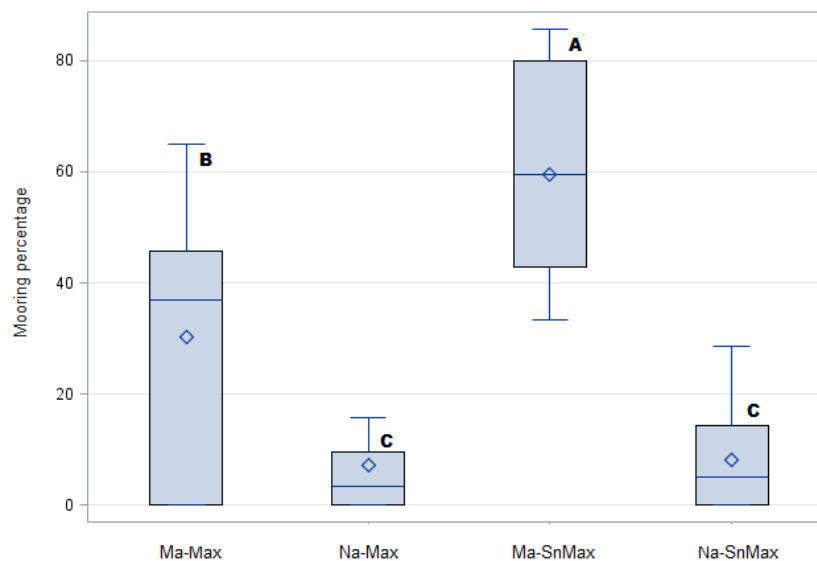
The data were analyzed using a randomized block design with a factorial arrangement, considering the factors of pollination type, pruning percentage, and fungicide application. Analysis of variance (ANOVA) and mean comparisons were performed with Tukey's test ( $\alpha \leq 0.05$ ) using the statistical package SAS (Statistical Analysis System).

## Results and Discussion

### Fruit mooring percentage with pollination and fungicide application

Trees subjected to hand-pollination without fungicide exhibited a statistically higher fruit set, averaging 59.58 %. This was followed by the treatment involving hand pollination with fungicide, which showed an average fruit set of 30.24 %. Trees undergoing natural pollination, both with and without fungicide, displayed a lower fruit set at 7.61 % on average, with no statistically significant differences (Figure 1).

Hand-pollination resulted in an 83.04 % higher fruit moorage compared to naturally pollinated trees. In terms of fungicide application, the hand-pollination treatment without fungicide showed a 49.24 % improvement in the fruit set compared to the hand-pollination treatment with fungicide. Sánchez-Monteón et al. (2019) stated that the low percentage yield or fruit set of hand-pollinated fruit could be due to inadequate flower selection during pollination (lack of receptivity), resulting in the absence of fertilization. Contrary to this, the results of this research indicate that flower selection was adequate, leading to a higher fruit set. Although anthracnose has been reported as one of the main diseases affecting inflorescences (Betancourt-Aranguré *et al.*, 2019), during the experiment, flowers not treated with fungicides exhibited a better fruit set.



**Figure 1. Percentage of fruit moorage (fruit set) with different types of pollination and fungicide use on soursop flowers.**

Means with a common letter are not significantly different ( $p > 0.05$ ).

Ma: Hand-pollination; Max: Maxtrobryn; Na: Natural pollination; Sn: Without maxtrobryn

## Percentage of fruit set with pruning and fungicide application

The percentage of fruit set with pruning and fungicide application was statistically significant among various treatments. Trees treated with fungicide (Max), hand-pollination (Ma) with pruning at 100 %, 75 %, and 50 %, as well as trees with natural pollination (Na) at pruning levels of 75 % and 25 %, showed a significantly higher fruit set at 24.60 %, compared to hand-pollination treatments with 25 % and 0 % pruning levels and natural pollination with 100 %, 25 %, and 0 %, which did not yield any fruit (0 %) (Figure 2).

Martinez & Vidal (1993) reported a 65 % fruit set with hand-pollination without the use of fungicide, while Nakasone & Paul (1998) reported up to 80 % fruit set with hand-pollination without fungicide. In soursop, fruit abortion or drop is attributed to anthracnose, which can cause up to 90 % fruit abortion or drop if environmental conditions favor its development (high relative humidity, poor aeration, and low light penetration in the tree) (Cambero-Ayón et al., 2019). Betancourt-Aranguré et al. (2019) reported that the use of Maxtrobyn (Azoxystrobin) can inhibit the development of anthracnose in vitro in inflorescences by up to 71.60 % with a dose of 0.88 mL. Meanwhile, Hernandez-Guevara & Lopez-Rodriguez (2019) reported a 67.50 % control of anthracnose on soursop leaves using Maxtrobyn (Azoxystrobin) and Flutriafol at a dose of 1200 mL ha<sup>-1</sup>.

In fruit trees, pruning is one of the most relevant agronomic factors, enabling greater aeration and light penetration in the tree, and preventing conditions conducive to the development of pests and diseases such as anthracnose. Additionally, pruning helps create a desired canopy with load support (Cruz-Barrón, 2011). Alternative methods, such as the use of microorganisms, have also been explored. Anaya-Martínez (2022) applied rhapsody (*Bacillus subtilis*) at a dose of 17.5 cm<sup>3</sup> L<sup>-1</sup> water, achieving a 70 % decrease in anthracnose incidence in soursop fruits and a 58.7 % control efficiency. This alternative proves to be environmentally favorable.

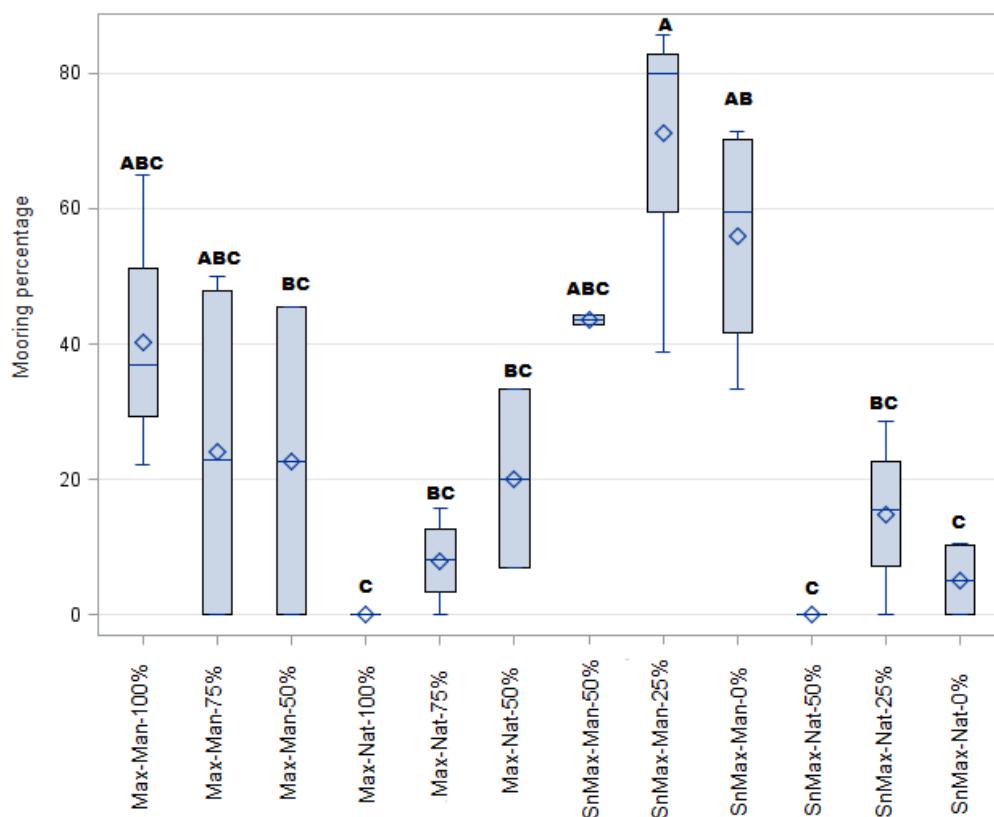
## Percentage of fruit moorage with pruning and without fungicide application

Trees without fungicide application (SnMax), manual pollination (Man) with pruning at 50, 25, and 0 %, as well as trees with natural pollination (Nat) with pruning at 25 and 0 %, were statistically significant, showing higher fruit moorage (38.12 %) compared to manual pollination treatments with 100 % and 75 % pruning and natural pollination with 100 %, 75 %, and 50 %, which did not moor the fruit (0 %) (Figure 2).

The results obtained in fruit moorage are highly variable, as some authors report a high percentage of fruit moorage, while others report a low percentage. With the manual pollination technique, Nakasone & Paul (1998) reported up to 80 %; Franco-Mora *et al.* (2001) recorded values of 70 to 30 %, and Martinez & Vidal (1993) reported values of 65 % fruit moorage. Meanwhile, Rubi (1994) found 8 % moorage in cherimoya under natural pollination conditions.

Percentages of fruit moorage have also been evaluated with hormone application. Guaycha-Armijos (2020), with the application of eco-hormones (750 mL ha<sup>-1</sup>), accelerated flower production (in 4 days) and formation of the first fruits (in 6 days) and decreased flower abortion by 25.26 % compared to the control (without hormones). Chávez & Sabando (2022), with the use of manual pollination, recorded an average of 73.33 % in the fruit set, and with the application of naphthaleneacetic acid at 2 %, recorded a maximum average of 63.33 %. This high percentage of fruit set represents an alternative to increasing yields in the orchards of this Anonacea.

The abortion or low fruit moorage is attributed to a deficiency in pollination or a blockage in the sexual process, as pollination may occur before the pollen grains germinate and fertilize the ovary (Escobar *et al.*, 1986; Nakasone & Paull, 1998). It is also important to mention that pollination and fertilization in soursop are limited by the characteristic phenomena of its flower (Worrell *et al.*, 1994). Temperature and relative humidity conditions are important during the pollination process; in dry places, the drying of the stigmatic liquid occurs faster, a situation that makes the germination of pollen grains on the stigma, the growth of the pollen tube, fertilization, and later the moorage impossible (Franco-Mora *et al.*, 1999; Nakasone & Paull, 1998; Rebolledo *et al.*, 2009; Cárdenas-Torres, 2002).



**Figure 2. Percentage of fruit set in trees with different pollination and pruning intensity. With and without Maxtrobyn (Azoxystrobin) fungicide application.**

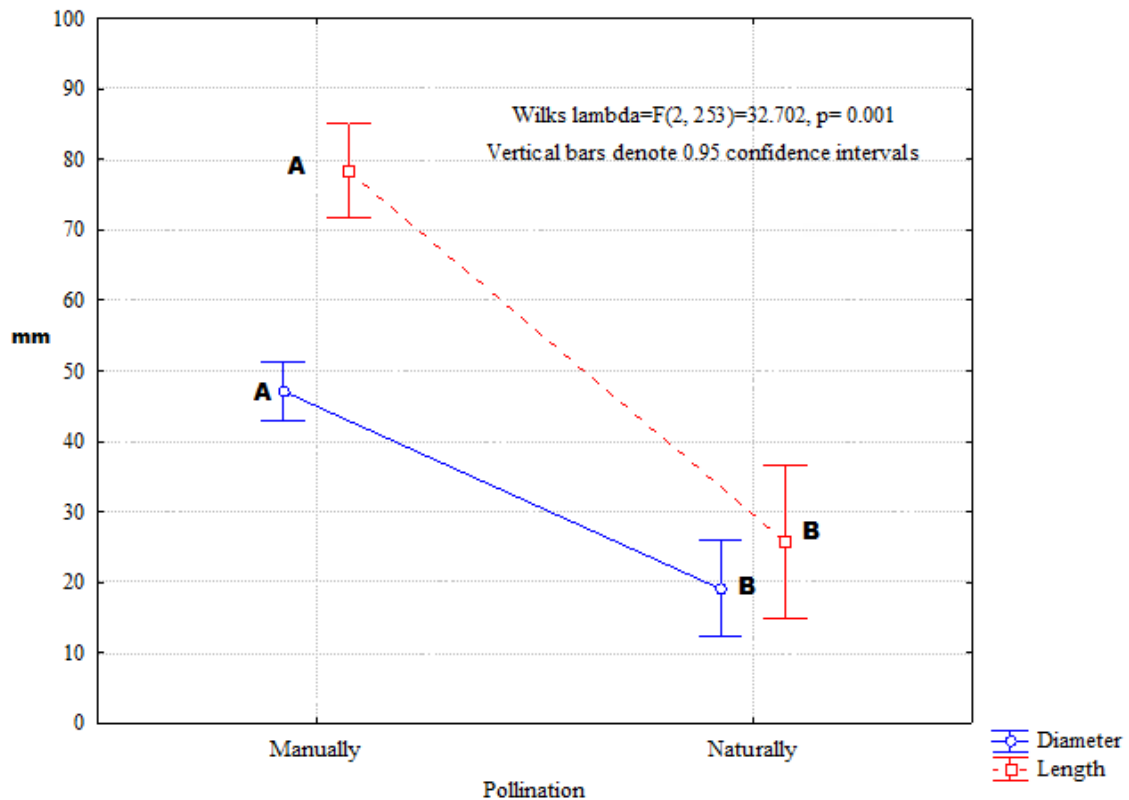
Means with a common letter are not significantly different ( $p > 0.05$ ).

Max: Maxtrobyn, SnMax: No Maxtrobyn; Man: hand-pollination; Nat: Natural pollination.

### Fruit development with different pollination types

Hand-pollinated fruits were statistically significant, presenting a greater diameter (46.75 mm) and length (78.76 mm) compared to naturally pollinated fruits with means of 21.86 mm in diameter and 29.29 mm in length (Figure 3). Hand-pollinated fruits were found to be 53.23 % and 62.80 % larger in diameter and length development, respectively. Hand-pollination increases tree production and fruit quality by up to 50 % (Oliveira *et al.*, 2005). Chávez & Sabando 2022), with the use of hand-pollination and the application of 2 % naphthaleneacetic acid, obtained diameters of 2.019 cm.



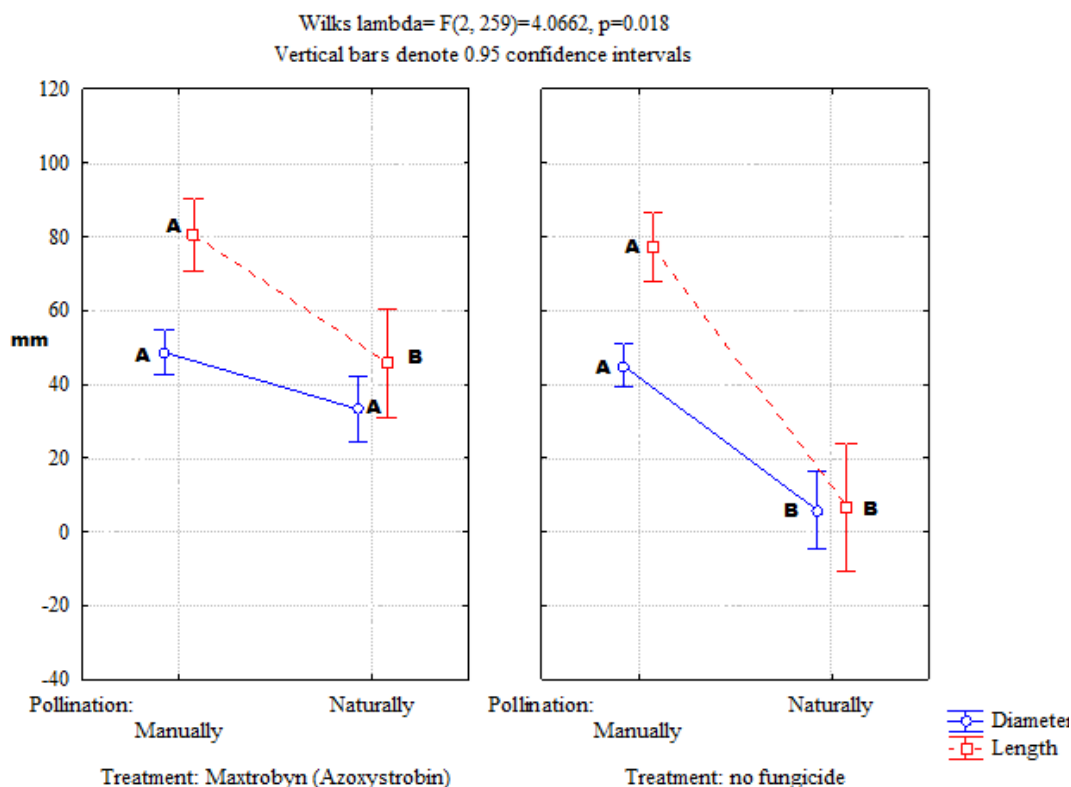


**Figure 3. Fruit diameter and length with hand- and natural pollination at Las Varas, Compostela, Nayarit.**

Means with a common letter are not significantly different ( $p > 0.05$ ).

### Fruit development with the use of fungicides and different pollination

Fruits treated with fungicide and subjected to hand-pollination demonstrated statistically greater size, with means of 48.64 mm in diameter and 80.48 mm in length. In contrast, fruits without fungicide and undergoing natural pollination exhibited smaller sizes, with means of 5.90 mm and 6.60 mm in diameter and length, respectively (Figure 4). In fruit development, it was observed that the treatment with hand-pollination and fungicide was 87.87 % larger in diameter and 93.91 % in fruit length. Betancourt-Arangur  *et al.* (2019) mentioned that the use of Maxtrobryn (Azoxystrobin) can inhibit the development of anthracnose *in vitro* in inflorescences by up to 71.60 % with a dose of 0.88 mL. Meanwhile, Hernandez-Guevara & Lopez-Rodr guez (2019) reported a 67.50 % control of anthracnose on soursop leaves with the use of Maxtrobryn (Azoxystrobin) and Flutriafol at a dose of 1200 mL ha<sup>-1</sup>.



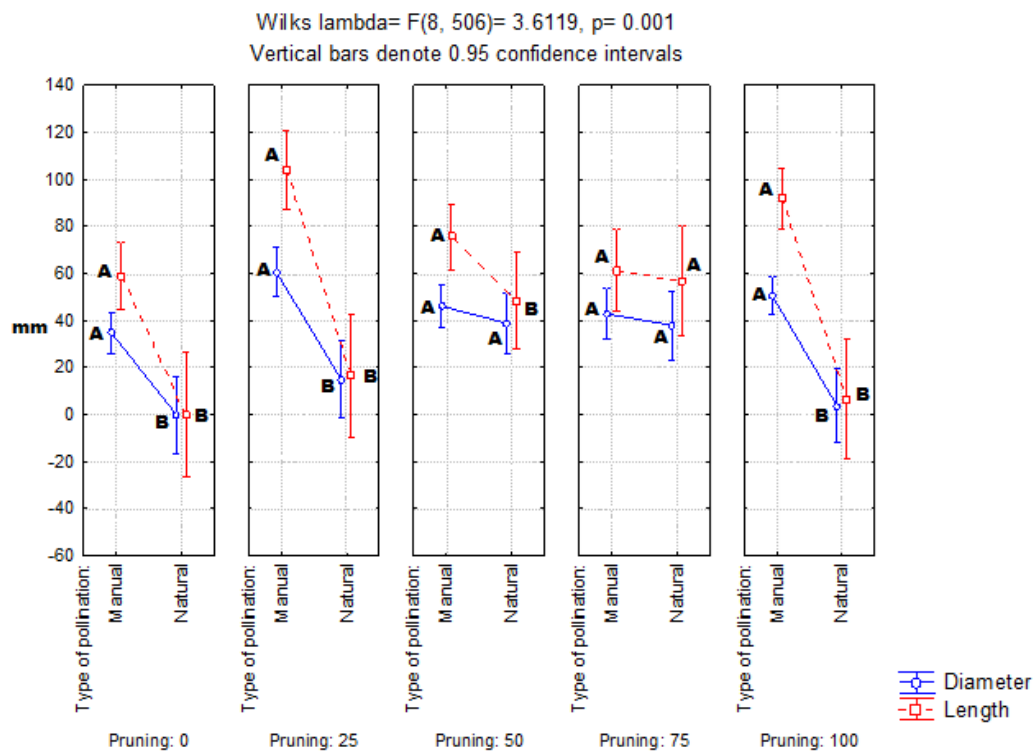
**Figure 4. Fruit diameter and length with hand- and natural pollination with the use of fungicide during the pollination process.**

Means with a common letter are not significantly different ( $p > 0.05$ ).

### Fruit development with different pruning and pollination intensity

Fruit development varied according to the intensity of pruning and pollination. In the treatment with 0 % pruning and manual pollination, the fruit presented an average diameter of 34.99 mm and a length of 59.05 mm; on the contrary, there was no mooring with natural pollination. The treatment with 25 % pruning and hand-pollination was outstanding in fruit development, with a diameter of 60.62 mm and a length of 103.88 mm. The treatments with 50 % pruning and hand-pollination, as well as natural pollination, were not significant in fruit diameter, with an average of 42.53 mm; however, fruit length was greater with hand-pollination, reaching 75.69 mm. The treatment with 75 % pruning and hand- and natural pollination was not significant, with an average fruit diameter of 44.22 mm and a length of 59.20 mm. The treatment with 100 % pruning and hand-pollination showed better fruit development, with a diameter of 50.86 mm and a length of 91.89 mm, in contrast to fruits with natural pollination, which showed a diameter of 3.69 mm and a length of 6.61 mm (Figure 5).

Nolasco *et al.* (2019) reported a mean of 21.82 cm in longitudinal diameter and 117.96 mm in equatorial diameter in harvested fruits, while Avila *et al.* (2012) reported a mean of 24 cm in longitudinal diameter and 41 cm in equatorial diameter.



**Figure 5. Equatorial and longitudinal diameter of hand-pollinated and naturally pollinated fruit on trees with different pruning intensities.**

Means with a common letter are not significantly different ( $p > 0.05$ ).

## Conclusions

The hand-pollination technique promoted a 31.64 % increase in soursop fruit mooring. The combination of Maxtrobyn fungicide application and different pruning intensities (100 %, 75 %, and 50 %) increased the fruit set by 28.97 %. Similarly, the interaction between manual pollination and different pruning intensities (100 %, 25 %, and 0 %) showed greater fruit size, with means ranging from 34.94 to 60.62 mm in diameter and 59.05 to 103.88 mm in length.

## Recommendation

Considering the environmental implications of the prolonged use of chemical products as a natural resource conservation strategy, it is advisable to evaluate biological controllers of these pathogens in the soursop crop.

## Authors contribution

Conceptualization of the work (LEG, CCJ, CCA, VHMV, BZCC, RRJ). Methodology development (BZCC, RRJ). Software management (BZCC, VHMV). Experimental validation (LEG, CCJ). Results analysis (BZCC, VHMV). Data management (BZCC, VHMV). Manuscript writing and preparation (BZCC, RRJ). Writing, revising, and editing (LEG, CCJ, CCA, VHMV). Project manager (LEG, CCJ). Acquisition of funds (LEG, CCJ).

All authors of this manuscript have read and accepted the published version of the manuscript.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

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