Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-8621
Doi: 10.4025/actasciagron.v45i1.62036

CROP PRODUCTION

Effect of plant-growth regulators, adjuvants and artificial pollination on the fixation and quality of *Annona squamosa* L cultivars' fruit

Pollyana Cardoso Chagas[1], Jonathan Crane[2], Edvan Alves Chagas[3*], Wagner Vendrame[4], Barbara Nogueira Souza Costa[5], Aurélio Rubio Neto[6] and Elias Ariel de Moura[1]

[1]Universidade Federal de Roraima , Campus Cauamé, Boa Vista, Roraima, Brazil. [2]Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, United States of America. [3]Empresa Brasileira de Pesquisa Agropecuária, Embrapa Rondônia, BR-174, km 8, Distrito Industrial, 69301-970, Boa Vista, Roraima, Brazil. [4]Environmental Horticulture Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, United States of America. [5]Florida Internacional University, Miami, Florida, United States of America. [6]Instituto Federal Goiano, Pólo de Inovação, Rio Verde, Goiás, Brazil. *Author for correspondence. E-mail: edvan.chagas@embrapa.br

ABSTRACT. The aim of the study was to assess the effect of plant regulators (gibberellic acid and naphthalene acetic acid) and adjuvant LI 700® with and without hand pollination on the fruiting and quality of *Annona squamosa* L. cultivars. The experiment was conducted in the experimental orchard of the Florida Tropical Research and Education Center (TREC/UF) in Homestead, Florida, USA. The experimental design in random blocks comprised 14 treatments, 10 repetitions and 3 flowers per treatment and per tree for 'Red' and 'Lessard Thai' cultivars. To assess quality, 9 fruit from each treatment were used, with 3 repetitions and 3 fruit per repetition. High instability was observed in fruit fixation rates between cultivars. The application of growth regulators did not increase fruit fixation in the tested cultivars. The application of naphthalene acetic acid had a deleterious effect on the flowers of the sugar apple cultivars. The most uneven (asymmetrical) fruit was obtained in the treatments with the application of growth regulators. The application of the adjuvant showed toxicity, causing damage to the skin of the fruit. The fruit with the greatest symmetry had the lowest levels of soluble solids and the highest toxicity. The application of growth regulators associated with hand pollination was not efficient, presenting a low percentage of fruit fixation for *Annonaceae* cultivars. None of the studied treatments produced parthenocarpic fruit (without seeds). The utilization of an adjuvant in the applied concentration caused damage to the fruit peels (darkening) due to toxicity in both cultivars.

Keywords: fruit quality; gibberellic acid; hand pollination; naphthalene acetic acid; sugar apple cultivars.

Received on January 5, 2022.
Accepted on April 11, 2022.

Introduction

The sugar apple tree (*Annona squamosa* L.) originated in Central America, probably in the Antilles region, and is considered one of the main representatives of the Annonaceae family. Different varieties of sugar apple trees have been utilized by the region's producers, among which 'Red' and 'Lessard Thai' are outstanding.

A. squamosa L. presents protoginic dichogamy; that is, the pistils mature before the stamens, limiting the occurrence of self-fertilization (Araújo et al., 2021; Kishore, Shukla, Babu, Sarangi, & Patanayak, 2012; Lau, Pang, Ramsden, & Saunders, 2017; Meade & Parnell, 2018). Although it produces large amounts of flowers at each production cycle, it is estimated that 5 to 10% of fruit are formed (Peña, Nadel, Barbosa-Pereira, & Smith, 2002), and this variation can change, depending on the number of visits and species of pollinator from the Nitidulidae family (Kishore et al., 2012). Moreover, fruit from natural pollination is generally small and malformed (asymmetric), which justifies the use of artificial pollination (Mota Filho et al., 2012; Moura et al., 2019; Moura et al., 2021; Pereira et al., 2019; Santos et al., 2019).

Artificial pollination increases the size, regulates the shape and improves several other physical and chemical characteristics of the fruit (Mota Filho et al., 2012). The benefits of the artificial pollination technique in species from the Annonaceae family have been emphasized in several works; however, the large number of seeds present in the fruit and the high costs associated with the work are the main disadvantages (Pereira et al., 2019; Santos et al., 2019).

However, studies have shown that fruit fixation can be promoted by applying auxins, gibberellins and cytokinins, without the need for pollination (Pereira, Crane, Montas, Nietsche, & Vendrame, 2014a; Verotti et al., 2019; Santos et al., 2016; Santos et al., 2019). One advantage of plant growth regulators in the production of fruit by parthenocarpy is the absence of seeds, which is one of the characteristics most appreciated by consumers (Kapłan, 2011).

Plant growth regulators are involved in the induction of fruit fixation and growth and act in elongation and cell division (Dorcey, Urbez, Blázquez, Carbonell, & Perez-Amador, 2009; Ramezani & Shekafandeh, 2009). Many studies assessing the effect of growth regulator applications in species of the Annonaceae family have demonstrated this plant growth regulators' capacity to increase fixation and produce parthenocarpic fruit (Pereira et al., 2014a; Pereira, Santos, Nietsche, Mizobutsi, & Santos, 2014b; Santos et al., 2016; Santos et al., 2019).

However, only 8% fixation of 'Gefner' atemoya fruit was observed when 450 mg L^{-1} of naphthalene acetic acid was applied 148 days after the first application, while Pereira et al. (2014a) observed 87% fruit fixation using the same dose in 'Gefner' atemoya. This controversial effect may have occurred due to the date when the naphthalene acetic acid treatment was applied, the inhibition of pollen grain germination and/or interruption of pollen tube growth (Reig, Mesejo, Martínez-Fuentes, & Agustí, 2014; Yang, Wang, Fu, Deng, & Tao, 2012), as has been observed in other cultures (Chouza, Gravina, & Borges, 2011; Reig et al., 2014).

Adjuvants are commonly used in agriculture to enhance the effectiveness of agrichemicals, such as fertilizers, herbicides and plant growth regulators (Czarnota & Thomas, 2015; Hazen, 2000). Polyoxyethylene alkyl ethers are nonionic surfactants made of an alkyl chain with methylene groups and a hydrophilic part with oxyethylene units. Nonionic surfactants are generally useful in enhancing the uptake and/or prolonging the efficacy of agrichemicals. However, we are not aware of any reports on their use in conjunction with plant growth regulators in the pollination of Annonaceae fruit.

In this regard, due to the high labor cost, long time spent executing artificial pollination, and low fixation and fruit quality, efforts have been made to replace artificial pollination in the cultivation of sugar apples using growth regulators. Therefore, the present study aimed to assess the effect of growth regulator application (gibberellic acid and naphthalene acetic acid) and adjuvants associated with artificial pollination on the fixation and quality of fruit in *Annona squamosa* L. cultivars.

Material and methods

Experimental conditions and experimental location

The experiment was conducted in an experimental sugar apple (*Annona squamosa* L.) orchard, with the cultivar 'Lessard Thai', in Homestead, Florida, USA at the Florida Tropical Research and Education Center (TREC/UF), (25°30'40.809" N, 80°30'3.983" W), with an altitude of 3.8 m and a subtropical climate. The location mean annual precipitation is 1,490 mm, based on meteorological data collected in TREC by the Florida Automated Weather Network (http://fawn.ifas.ufl.edu/), with most rainfall (70%) concentrated from late May until early November. The soil is clayey (Noble, Drew, & Slabaugh, 1996), well drained, with overlapping limestone layers and the presence of rocks.

Plant material and experimental design

The experiment was installed on February 10, 2018. Ten plants of 'Red' and 'Lessard Thai' sugar apple cultivars were selected, considering uniformity, force and soundness. The plants were 18 years old and cultivated in a 6 x 4 m spacing. They were mechanically and manually pruned on the second fortnight of February 2018. The irrigation system uses conventional spraying twice a week for four hours a day. Invasive plants were controlled between rows with a mechanical trimmer every two months, and canopy projections and chemical control were used in the plantation line. Fertilization was conducted according to soil analysis and culture demand after production pruning.

The experimental design was random blocks with 14 treatments and 10 repetitions, with 3 flowers per portion, identified with colored plastic tags, and to each plant of each cultivar, treatments described in Table 1 were applied.

For treatments with hand pollination (HP), flowers in the male stage were collected in the morning at 6 o'clock to collect pollen grains. The collected pollen grains were placed in Eppendorf microtubes. Then, with brush number 6, the pollen was deposited on the stigma of flowers in the female stage, which were previously identified in each treatment.

Table 1. Treatments applied to flowers at anthesis and after anthesis in sugar apple cultivars 'Lessard Thai' and 'Red'.

Treatments	NAA[z]	GA_3[z]	LI 700 Adjuvant[z]	Hand Pollination	Period of application of the solutions
	mg 100 L^{-1}				
T1	0	0	No	No	0
T2	0	0	No	Yes	0
T3	0	0	Yes	No	Female stage
T4	0	0	Yes	Yes	Female stage
T5	450	750	Yes	Yes	Female stage, 1 and 3 WAA
T6	450	1000	Yes	Yes	Female stage, 1 and 3 WAA
T7	450	1250	Yes	Yes	Female stage, 1 and 3 WAA
T8	450	1500	Yes	Yes	Female stage, 1 and 3 WAA
T9	450	750	Yes	No	0
T10	450	1000	Yes	No	0
T11	450	1250	Yes	No	0
T12	450	1500	Yes	No	0
T13	450	1000	No	No	0
T14	450	1000	No	Yes	Female stage, 1 and 3 WAA

z, WAA, weeks after anthesis; NAA, naphthalene acetic acid; GA_3, gibberellic acid; ppm, parts per million; LI 700 surfactant at 1.5% of the commercial rate.

The following commercial products were used: PoMaxa (Valente BioSciences Corporation Technology Way, Libertyville, IL, USA) with 3.1% of 1-naphthalene acetic acid (NAA); ProGibb LV Plus (Valente BioSciences Corporation Technology Way, Libertyville, IL, USA) with 5.7% gibberellic acid (GA_3) and Adjuvant LI 700 (Loveland Products, Greeley, CO, USA).

The solutions were prepared with dilutions of the commercial products in water using a volumetric flask. In treatments with the adjuvant, a 1.5% concentration of the commercial product was used. Later, they were stored in "spray" flasks at a temperature of 5°C in a refrigerator for 24 hours.

On May 9, 2018, during the flowering period, closed flowers (pre-anthesis stage) were selected, tagged according to each treatment and protected from pollinators with white paper bags. The following morning, the solutions were applied, directing them to the flowers during anthesis, that is, flowers in the female stage, and each flower received around 0.5 mL (2 sprinkles) othe stigma (Pereira et al., 2014a). In treatments without hand pollination, the flowers were once again protected with white paper bags, thus avoiding natural pollination. All treatments were applied on the same day. For treatments with plant regulators, two additional applications were made 7 and 21 days after anthesis.

Determination of the evaluated characteristics

Fruit fixation was assessed one week after anthesis and then every two weeks. Fruit harvest started in the 13th week after anthesis (92 days) and was made when they presented distance from the peel carpels and light pink color of tissues between carpels, as described by Moura et al. (2021).

After harvest, the following aspects were assessed: fruit mass (FM); peel + stalk mass (PSM); and seed mass (SM), utilizing a digital scale (±0.01 g), and results were expressed in grams (g). Fruit length (FL) and fruit diameter (FD) were assessed with a digital caliper (±0.01 g). The number of seed per fruit (NS) was assessed with manual counting. The fruit shape was assessed according to the classification proposed by Pereira et al. (2014a), where fully irregular fruit obtained grade 25%; partially irregular, grade 50%; regular, with little deformation, grade 75%; and fully regular (perfect round fruit), grade 100%.

During the experiment, toxicity symptoms were observed in some treatments; thus, the toxicity percentage was also calculated using a scale from 0% (fruit without toxicity symptoms) to 100% (fruit with toxicity symptoms across the epidermis). Later, the fruit was stored in the lab at a constant temperature of 25°C until ripening (horticultural maturity).

At ripening, 9 fruit samples from each treatment were assessed, with 3 repetitions and 3 fruit samples per repetition. The fruit was assessed as follows: pulp yield—calculated by subtracting PSM and SM from FM, expressed in percentage (%); soluble solids (SS), determined in digital refractometer, with values expressed in °Brix (Association of Official Agricultural Chemists [AOAC], 2012); pH (hydrogen-ion potential); and titratable acidity (TA), determined by titrimetry with sodium hydroxide solution (0.1 N) using Compact Titrator G20S (Mettler Toledo®), and the results were expressed in grams of citric acid 100 g^{-1} of pulp (AOAC, 2012). The ratio was calculated by the relationship between SS/TA contents.

Statistical analysis

Data were tested for normality according to the Shapiro–Wilk test and for homogeneity of variances according to Bartlett test ($p < 0.05$). Since normality and homogeneity assumptions were met, data were submitted to two-way analysis of variance by the F test ($p < 0.05$), followed by the Tukey test ($p < 0.05$). The relationship between the evaluated parameters was estimated by considering the Pearson correlation coefficient ($p < 0.05$). Multivariate data analysis was performed using principal component analysis (PC). All statistical analyses were performed using R software (R Core Team, 2020). For values outside the normality and homogeneity standards, the data were transformed using $(x+1)^{0.5}$.

Results

Fruit fixation

The sugar apple cultivars presented a good percentage of fruit fixation, with more than 90% for 'Lessard Thai' and 73% for 'Red', in all treatments using growth regulators (GR) until the 1st week after anthesis (WAA). For both cultivars, fruit fixation was below 10% in treatments without GR and without hand pollination (HP). The cultivars presented stability in fruit fixation in the 7th week for 'Red' and in the 9th week for 'Lessard Thai' (Table 2).

Table 2. The percentage of fruit set of the sugar apple cultivars 'Lessard Thai' and 'Red' after treatment with or without growth regulators (NAA and GA_3) and LI 700 adjuvant and with or without hand pollination.

*Treatments	Weeks after anthesis (WAA)						
	1	3	5	7	9	11	13
	Fruit Set (%)						
Cultivar 'Lessard Thai'							
1	10	3	3	3	3	3	-
2	97	97	97	97	97	97	-
3	10	3	3	3	3	3	-
4	63	47	40	40	40	40	-
5	97	80	67	63	63	63	-
6	93	83	63	53	53	53	-
7	90	80	63	60	57	57	-
8	97	90	67	57	57	57	-
9	97	37	3	3	3	3	-
10	97	47	3	0	0	0	-
11	100	63	13	7	7	7	-
12	90	53	13	7	7	3	-
13	97	67	7	3	3	3	-
14	97	80	47	47	47	47	-
CV (%)	39	50	92	100	100	102	-
Cultivar 'Red'							
1	7	3	0	0	0	0	0
2	43	40	37	33	33	33	33
3	3	0	0	0	0	0	0
4	40	33	33	30	30	30	30
5	100	73	40	40	40	40	40
6	100	83	67	57	57	53	53
7	97	77	57	53	53	50	50
8	100	80	63	47	47	43	43
9	80	40	10	7	7	7	7
10	83	43	3	0	0	0	0
11	73	53	10	0	0	0	0
12	87	43	17	7	7	7	7
13	97	33	3	0	0	0	0
14	100	63	33	30	30	30	30
**CV (%)	48	55	90	101	101	99	99

*Treatments applied to flowers at anthesis and after anthesis of the sugar apple cultivars 'Lessard Thai' and 'Red' are described in Table 1. **coefficient of variation.

'Lessard Thai' presented an excellent final fruit fixation in treatment 2 (HP), with good stability, and 97% of fruit was fixed from the 1st to the 11th WAA, a very relevant characteristic considering the morphophysiological aspects of the flowers (Table 2). The same was not observed for the 'Red' cultivar, which presented instability in the period of fruit development, fixing only 33% of fruit. Treatments with GR (GA_3

and NAA) associated with HP obtained fruit fixation values ranging from 47 to 63% (11th WAA) for the 'Lessard Thai' cultivar, and variation between 30 and 53% (11th WAA) for the 'Red' cultivar. Without HP and with or without RG (NAA and GA_3), fruit fixation was very low, ranging from 3 to 7% for both cultivars, showing that GR application in the tested concentrations did not contribute to increased fruit fixation, which can be attributed to the deleterious effect of NAA on flowers (Table 2).

Symmetry and physical and physicochemical characteristics of fruit

When assessing production and physical and physical-chemical quality, treatments without HP (T1, T3 T9, T10, T11, T12, and T13) did not produce enough fruit for statistical analysis. For 'Red' and 'Lassard Thai' cultivars, fruit was more uniform (symmetrical) with HP (T2), with 91.66 and 94.44% symmetry, respectively (Table 3).

Table 3. Fruit shape and peel damage (toxicity) of the sugar apple cultivars 'Lessard Thai' and 'Red' fruit after hand pollination treatments with or without NAA and GA_3 and adjuvant.

*Treatments	Shape (%)	Toxicity (%)	Shape (%)	Toxicity (%)
	Cultivar 'Lessard Thai'		Cultivar 'Red'	
2	94.44 a	0 b	91.66 a	0 b
4	69.44 b	11.11 b	67.77 ab	19.44 b
5	77.77 ab	50.00 a	63.88 b	55.55 a
6	86.11 ab	50.00 a	72.22 ab	75.00 a
7	77.77 ab	44.44 a	72.22 ab	63.88 a
8	77.77 ab	33.33 a	72.77 ab	61.11 a
14	77.77 ab	0 b	69.44 ab	0 b
C.V. (%)	10.99	30.35	13.49	33.85

Means followed by the same letter in a column do not differ from each other by the Tukey test ($p < 0.05$). *Treatments applied to flowers at anthesis and after anthesis of the sugar apple cultivars 'Lessard Thai' and 'Red' are described in Table 1.

However, the most uneven fruit (asymmetrical) was observed in treatment 5 (450 mL L^{-1} NAA and 750 mL L^{-1} GA_3, with HP and Adjuvant), with 68.88% for 'Red,' and T4 (with HP and Adjuvant), with 69.44% for 'Lessard Thai' ($p < 0.05$). The application of adjuvant LI700 was harmful to fruit, causing toxicity (that is, darkening of peel surface) between 19.44 and 75% in 'Red' fruit peels and between 11.11 and 50% for 'Lessard Thai' fruit peels. Plants that did not receive adjuvant application presented fruit without damage to the peel (Table 3).

For 'Lessard Thai,' characteristics of length, diameter, seed mass, number of seeds and pulp percentage did not present significant differences across treatments ($p > 0.05$; Table 4).

Table 4. Physical fruit characteristics of the sugar apple cultivars 'Lessard Thai' and 'Red': Fruit Length-FL (mm), diameter-FD (mm), mass-FM (g), Peel + stem mass-PSM (g), seed mass-SM (g), number of seeds per fruit-NS, and percent of the pulp (%) after application of different treatments with or without growth regulators (NAA and GA_3) and the adjuvant and with hand pollination.

*Treatments	FL	FD	FM	PSM	SM	NS	Pulp
			Cultivar 'Lessard Thai'				
2	87.92 a	89.27 a	309.26 a	161.52 a	17.16 a	40.33 a	41.92 a
4	84.89 a	81.91 a	251.90 ab	111.38 b	18.00 a	43.33 a	47.45 a
5	77.21 a	79.69 a	215.00 b	109.22 b	14.37 a	35.22 a	42.28 a
6	83.05 a	83.28 a	259.31 ab	113.63 b	16.96 a	42.33 a	49.47 a
7	86.89 a	87.14 a	289.62 ab	135.88 ab	21.47 a	55.55 a	45.66 a
8	86.21 a	84.85 a	268.37 ab	113.08 b	29.9 a	48.77 a	46.10 a
14	76.70 a	78.71	216.01 b	101.35 b	15.26 a	40.55 a	45.34 a
C.V. (%)	4.99	4.98	12.73	12.49	18.78	20.04	10.88
			Cultivar 'Red'				
2	89.90a	100.62a	400.10a	206.35a	20.93a	45.33a	42.78 a
4	78.92ab	85.82ab	246.15bc	139.34abc	12.42abc	25.55ab	36.76 a
5	68.88b	69.79b	143.21c	71.06c	7.13c	17.44b	43.99 a
6	79.15ab	80.14b	230.72bc	115.87bc	11.08bc	27.00ab	44.95 a
7	72.95b	78.71b	204.84bc	104.88bc	7.44c	17.66b	44.36 a
8	85.03ab	85.95ab	288.32ab	148.03ab	16.72ab	41.00a	44.05 a
14	79.88ab	78.51b	219.05bc	115.75bc	11.93bc	29.44ab	38.76 a
C.V. (%)	7.22	4.46	20.28	19.38	24.3	26.96	8.72

Means followed by the same letter in a column do not differ from each other by the Tukey test ($p < 0.05$). *Treatments applied to flowers at anthesis and after anthesis of the sugar apple cultivars 'Lessard Thai' and 'Red' are described in Table 1.

The highest average fruit mass (FM) was obtained with HP (T2); however, it was statistically equal to the FM of treatments 4, 6, 7, and 8 ($p > 0.05$). This higher value is attributed to the PSM ($r = 0.88$, $p = 0.001$;

Figure 1), which also presented the highest average with HP (T2) and was not statistically different from T7 (450 NAA, 1,250 GA_3) ($p > 0.05$; Table 4). With regard to 'Red' cultivar physical fruit characteristics, treatment 2 (HP) had a higher FL (89.90 mm), FD (100.62 mm), FM (400.10 mm), PSM (206.35 mm), SM (20.93 mm), and NS (45.33) than fruit from other treatments (Table 4).

In the present research, parthenocarpic (without seeds) fruit production was not observed for treatments of 'Red' and 'Lessard Thai' cultivars, including those that received RG and were not pollinated (Table 3). Although the flowers were bagged at anthesis, this protection was probably not sufficient to avoid natural pollination by small beetles.

'Red' and 'Lessard Thai' cultivars did not present statistical differences across treatments for TA and Ratio (SS/TA), and only soluble solids (SS) for 'Lessard Thai' ($p > 0.05$). SS in the 'Red' cultivar obtained the highest content in treatment 8 (450 mg L^{-1} and 1,500 mg L^{-1}, with HP) with 25.16°Brix, but it was statistically different only from treatment 14, with 22.52°Brix ($p < 0.05$; Table 5).

Table 5. Physicochemical fruit characteristics of sugar apple cultivars 'Lessard Thai' and 'Red': Soluble solids—SS (°Brix), Hydrogen potential (pH), Titratable acidity—TA (g citric acid 100 g^{-1} pulp), and Ratio (soluble solids / Titratable acidity) after application of different treatments with or without growth regulators (NAA and GA_3) and adjuvant and with hand pollination.

*Treatments	SS	pH	TA	*Ratio*
		Cultivar 'Lessard Thai'		
2	22.27 a	4.86 b	0.21 a	105.24 a
4	23.34 a	5.05 a	0.22 a	110.49 a
5	24.01 a	5.02 a	0.19 a	130.70 a
6	22.66 a	4.99 ab	0.21 a	106.82 a
7	23.58 a	5.07 a	0.23 a	105.42 a
8	23.57 a	5.02 a	0.23 a	105.72 a
14	23.20 a	5.08 a	0.21 a	115.23 a
C.V. (%)	3.82	0.85	2	11
		Cultivar 'Red'		
2	23.54 ab	4.84 d	0.21 a	115.04 a
4	23.92 ab	5.07 ab	0.24 a	100.43 a
5	23.51 ab	5.18 a	0.19 a	128.03 a
6	24.43 ab	5.04 abc	0.20 a	127.69 a
7	23.92 ab	4.93 bcd	0.19 a	131.23 a
8	25.16 a	4.96 bcd	0.22 a	112.40 a
14	22.52 b	4.87 cd	0.21 a	110.21 a
C.V. (%)	2.93	1.01	1.67	10.47

Means followed by the same letter in a column do not differ from each other by the Tukey test ($p < 0.05$). *Treatments applied to flowers at anthesis and after anthesis of the sugar apple cultivars 'Lessard Thai' and 'Red' are described in Table 1.

For fruit pH, 'Red' and 'Lessard Thai' cultivars were more acidic (4.86 and 4.84, respectively) when treated with HP (T2), a characteristic that provides more sweetness to the fruit, with high rates of SS. (Table 5).

Multivariate analysis

Principal component analysis (PCA) explained 72.79% of data in the first two components for 'Red' and 'Lessard Thai' cultivars (Figure 1). PCA1 presented a variance of 56.99%, and 'Red' in treatments 2, 5 and 7 contributed the most, with 29.37, 30.66, and 12.15%, respectively. PCA2 explained 15.80% of the variance, and 'Red' in treatments 8 and 14 and 'Lessard Thai' in treatments 2, 7, and 8 contributed the most, with 12.54, 14.35, 21.98, 14.52, and 13.96%, respectively (Figure 1).

The variables that presented a higher positive correlation with PC1 were: FL ($r = 0.96$, $p < 0.0001$); FM ($r = 0.95$, $p < 0.0001$); FD ($r = 0.95$, $p < 0.0001$); PSM ($r = 0.86$, $p < 0.0001$); NS ($r = 0.80$, $p < 0.0005$); SM ($r = 0.78$, $p<0.0001$); Shape ($r = 0.77$, $p < 0.005$) and negatively, pH ($r = -0.59$, $p < 0.05$); Toxicity ($r = -0.54$, $p < 0.05$). For PC2, the variables with a high correlation were: SS ($r = 0.63$, $p < 0.01$), pH ($r = 0.60$, $p < 0.05$), and TA ($r = 0.57$, $p < 0.05$; Figure 1).

Shape and PSM were higher under HP treatment (T2). As already reported, the most uniform fruit (symmetrical) was that under HP (T2), which also presented a larger size. The highest fruit shape (symmetry) was fully correlated with PSM ($r = 0.70$, $p < 0.05$), and a larger PSM was a direct response of fruit development, which presented correlation to FL ($r = 0.77$, $p < 0.001$), FD ($r = 0.92$, $p < 0.0001$), and FM ($r = 0.95$, $p < 0.0001$; Figure 1).

However, the higher the fruit symmetry (shape), the lower the SS values ($r = -0.70$ $p = 0.005$) and the higher the toxicity ($r = 0.64$, $p < 0.05$; Figure 1). A high TA was directly correlated to a higher NS ($r = 0.56$ $p < 0.05$)

and SM (r = 0.61, $p < 0.05$), particularly with HP and application of 450 mg L^{-1} NAA + 1,250 mg L^{-1} GA_3 (T7, 'Lessard Thai') and 450 mg L^{-1} NAA + 1,500 mg L^{-1} GA_3 (T8, 'Lessard Thai'; Figure 1). 'Red' and 'Lessard Thai' in treatments 5 and 14 and 'Red' in treatments 4, 7, and 6 had smaller fruit with low organoleptic quality.

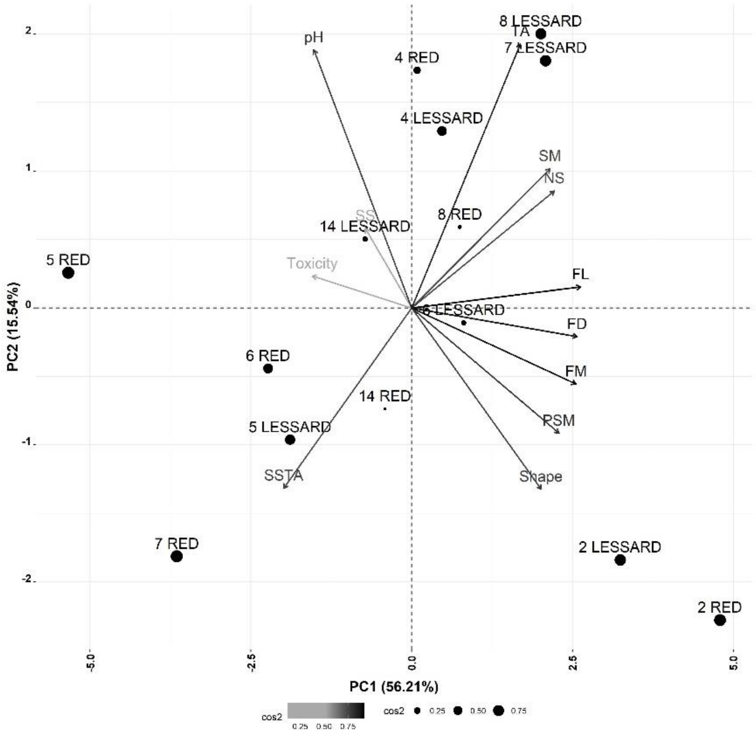

Figure 1. Principal component analysis (PCA) of 'Red' and 'Lessard Thai' cultivars with different characteristics. Treatments 2—'Red' and 'Lessard Thai (with HP and without adjuvant), 4—'Red' and 'Lessard Thai' (with HP and with adjuvant), 5—'Red' and 'Lessard Thai' (450 NAA + 750 GA_3 with HP and with adjuvant), 6—'Red' and 'Lessard Thai' (450 NAA + 1,000 GA_3 with HP and with adjuvant), 7—'Red' and 'Lessard Thai' (450 NAA + 1,250 GA_3 with HP and with adjuvant), 8—'Red' and 'Lessard Thai' (450 NAA + 1500 GA_3 with HP and with adjuvant), and 14—'Red' and 'Lessard Thai' (450 NAA + 1,000 GA_3 with HP and with adjuvant).

Discussion

Although *Annonas* sp. flowers are hermaphroditic, they present protoginic dichogamy and pollination syndrome, presenting specific flower characteristics that filter some specific pollinators (Kishore et al., 2012). Moreover, the low occurrence of natural pollinators in orchards hinders natural pollination (Costa, Silva, Paulino-Neto, & Pereira, 2017; Jenkins, Cline, Irish, & Goenaga, 2013; Jenkins et al., 2015). The absence of pollinators in orchards resulted in production below 5% of the plant's capacity (Peña et al., 2002). This low natural pollination occurs due to the number of visits and the species of pollinators. Kishore et al. (2012) reported that, among flowers with one visit, only 10% were pollinated; for flowers with five visits, over 35% were pollinated by *Carpophilos hemipterous*, while 22% of flowers were pollinated by *Carpophilos domidiatus*. Thus, among the techniques for culture management, hand pollination is definitely one of the most important, since self-fertilization is insignificant and the efficiency of *Nitidulidae* beetles is low for the *Annona* genus.

Although the practice of artificial pollination in sugar apple tree cultivation is fundamental for higher productivity and financial return for farmers, the demand for technical and intensive labor increases production costs. Thus, studies that seek new alternatives to increment sugar apple fruit production and quality, such as fruit production by parthenocarpy using growth regulators, are highly relevant.

In the present study, the low efficacy of fruit fixation (<63%) when associated with HP along with growth regulators makes us believe that NAA may have caused abortion of these plants' fruit (Table 3). According to Reig et al. (2014), NAA inhibits pollen grain germination and/or interrupts pollen tube growth, determining the development of new embryos depending on the treatment date. Although there are several studies confirming such responses in other cultures, including apple (Li, Li, & Shu, 1998), citrus (Chouza et al., 2011) and medlar (Reig et al., 2014), thus far, no studies have been performed on the effect of NAA on pollen germination and pollen tube growth, whether *in vitro* or *in vivo* for *Annona* species, and such studies are needed to identify the NAA mechanisms in flowers.

This response associated with NAA is more evident when we observe studies of *Annonaceae* using only GA_3. In studies with 'Gefner' atemoya, the following results were observed: 85% (Pereira et al., 2014b) and 50% (Pereira et al., 2014a) of fruit fixation using 1000 mg L^{-1} GA_3. Variations from 80 to 95% in fruit fixation with or without HP mixed with 500, 1,000, and 1,500 mg L^{-1} were observed by Santos et al. (2016). In contrast, studies using 450 mg L^{-1} NAA + 1,000 mg L^{-1} GA_3, (Mota Filho et al., 2012) obtained only 8% fruit fixation. However, Pereira et al. (2014b) observed 87% fruit fixation. This controversial effect may occur depending on the date of treatment with NAA (Reig et al., 2014; Yang et al., 2012).

This positive effect on atemoia hybrid cultivars (*A. cherimola* x *A. squamosa*) can be explained by crossing different species in which the hybrid acquires evolutionary characteristics with characteristics not presented by the parents. In addition, Martin, Viruel, Lora, and Hormaza (2019) showed a high rate of triploidy in atemoya hybrids and in the interspecific and intraspecific crosses of Anonaceae. These results lead to high variability in the genus *Annona*, causing different genotypes and phenotypes.

The high deformity of pollinated fruit occurred due to the non-fertilization of multiple ovaries caused by the poor deposition of the pollen grain or optimization of stigma exudate, which leads to variation in the number of seeds and generates irregular fruit (asymmetrical) (Lau et al., 2017; Li, Thomas, & Saunders, 2015; Meade & Parnell, 2018; Santos et al., 2019; Wang, Armbruster, & Huang, 2012). In the present experiment, an irregular fruit shape occurred due to the absence of seeds and pulp in some parts of the fruit, which affected the natural shape of the cultivar (Table 3). In treatments with the application of chemicals (adjuvant and/or growth regulators), the solution jet, at the moment of application, may have washed away some of the pollen grains deposited on the stigma, causing unevenness in the fruit. However, it is worth mentioning that the irregularity verified in fruit in treatments with growth regulators was less 23%, while Pereira et al. (2014b) observed 27% deformation in 'Gefner' atemoya fruit with the application of gibberellic acid.

The use of a growth regulator associated with hand pollination did not contribute to higher growth of 'Lessard Thai' sugar apple fruit and presented results similar to those of HP (T2; Table 4). Similar results were observed by Mota Filho et al. (2012), who did not obtain differences in fruit size by applying 450 mg L^{-1} NAA and 1 g L^{-1}, and by Verotti et al. (2019), who did not observe fruit size differences after applying mixes of growth regulators (auxin, cytokinin and gibberellin). Mahorkar, Naglot, Yadav, and Hake (2020) observed statistical differences for fruit size (length and diameter), with 75 mg L^{-1} of GA_3 for the largest fruit size but with smaller fruit (71.2 mm FL and 72.4 mm FD) than those observed in the present study.

As reported, a larger fruit size depends exclusively on the fertilization of multiple ovaries, thus increasing the number of seeds and, consequently, the auxin content required for fruit development. Furthermore, as reported by Mahorkar et al. (2020), the increase in the level of carbohydrates and GA_3 may have stimulated cell division and cell elongation, resulting in a larger fruit size. Larger fruit is desirable in commercial orchards since producers are better remunerated when they trade larger fruit.

In the present study, parthenocarpic fruit (without seeds) production was not observed for the tested treatments (Table 4). Even in treatments with growth regulators and without hand pollination (T9, T10, T11, T12, and T13), the presence of seed was observed (however in smaller quantities) when fruit was produced. Although the flowers were bagged during anthesis, apparently this protection was not efficient in avoiding natural pollination by small beetles from the *Nitidulideae* family. However, studies that used growth regulators to obtain parthenocarpic fruit in species of the Annonaceae family showed a gibberellin capacity to increase set and produce fruit without seeds, which indicates partial replacement of the role played by the pollen grain in the moment of pollination and fertilization by this growth regulator (Koura, Hasegawa, Yamamoto, & Yonemoto, 2004; Mota Filho et al., 2012).

In general, the application of plant regulators did not affect fruit quality. Similar results were observed by Pereira et al. (2019) with Annonaceae cultivars. In contrast, Verotti et al. (2019) observed SS increases (°Brix) and pH reduction with the increase in concentration of plant regulator mix (auxin, cytokinin and gibberellin). The produced fruit presented a standard organoleptic quality for the species, with physical and physicochemical characteristics similar to those of other studies (Moura et al., 2019; 2021; Pereira et al., 2014a; 2014b; Pereira et al., 2019; Santos et al., 2016; 2019).

Conclusion

The application of growth regulators associated with hand pollination proved to be inefficient, presenting a low percentage of fruit fixation for Annonaceae cultivars. None of the studied treatments produced parthenocarpic fruit (without seeds). New methodologies that use growth regulators at different concentrations and times should be conducted. The use of adjuvants in the concentration applied caused damage to the peel (darkening) of fruit due to toxicity in the two cultivars. There were high rates of abortion in flowers treated with NAA, and new studies should be conducted to analyze NAA behavior in pollen grain germination in *A. squamosa* L. cultivars.

Acknowledgements

The authors thank the Higher Education Personnel Improvement Coordination (CAPES) for their scholarships and National Council for Scientific and Technological Development (CNPq) for correspondent author's Productivity Research Scholarship.

References

Association of Official Agricultural Chemists [AOAC]. (2012). *Official methods of analysis of AOAC International* (19th ed.). Gaithersburg, MD: AOAC International.

Araújo, D. C., Chagas, P. C., Chagas, E. A., Moura, E. A., Oliveira, R. R., Taveira, D. L. L., ... Grigio, M. L. (2021). Flower stages, germination and viability of pollen grains of *Annona squamosa* L. in tropical conditions. *Acta Scientiarum. Technology*, *43*(1), 1-10. DOI: https://doi.org/10.4025/actascitechnol.v43i1.51013

Chouza, X., Gravina, A., & Borges, A. (2011). Control de la autopolinización, germinación del polen y crecimiento del tubo polínico en mandarina 'Montenegrina'. *Agrociencia Uruguay*, *15*(1), 27-36.

Costa, M. S., Silva, R. J., Paulino-Neto, H. F., & Pereira, M. J. B. (2017). Beetle pollination and flowering rhythm of Annona coriacea Mart. (*Annonaceae*) in Brazilian cerrado: Behavioral features of its principal pollinators. *PLoS ONE*, *12*(2), 1-14. DOI: https://doi.org/10.1371/journal.pone.0171092

Czarnota, M., & Thomas, P. (2015). *Using surfactants*, *wetting agents*, *and adjuvants in the greenhouse*. Retrieved on Dec. 10, 2021 from https://extension.uga.edu/publications/detail.html?number=B1319

Dorcey, E., Urbez, C., Blázquez, M. A., Carbonell, J., & Perez-Amador, M. A. (2009). Fertilization-dependent auxin response in ovules triggers fruit development through the modulation of gibberellin metabolism in Arabidopsis. *The Plant Journal*, *58*(2), 318-332. DOI: https://doi.org/10.1111/j.1365-313X.2008.03781.x

Hazen, J. L. (2000). Adjuvants—terminology, classification, and chemistry. *Weed Technology*, *14*(4), 773-784. DOI: https://doi.org/10.1614/0890-037X(2000)014[0773:ATCAC]2.0.CO;2.

Jenkins, D. A., Cline, A. R., Irish, B., & Goenaga, R. (2013). Attraction of pollinators to atemoya (*Magnoliales: Annonaceae*) in Puerto Rico: a synergistic approach using multiple Nitidulid lures. *Journal of Economic Entomology*, *106*(1), 305-310. DOI: https://doi.org/10.1603/EC12316

Jenkins, D. A., Millan-Hernandez, C., Cline, A. R., McElrath, T. C., Irish, B., & Goenaga, R. (2015). Attraction of pollinators to atemoya (*Annona squamosa* × *Annona cherimola*) in Puerto Rico using commercial lures and food attractants. *Journal of Economic Entomology*, *108*(4), 1923-1929. DOI: https://doi.org/10.1093/jee/tov136

Kapłan, M. (2011). The effect of the method of application of growth regulators on fruit quality of 'Einset Seedless' grape (*Vitis* sp. L.). *Acta Agrobotanica*, *64*(4), 189-196. DOI: https://doi.org/10.5586/aa.2011.060

Kishore, K., Shukla, A. K., Babu, N., Sarangi, D. N., & Patanayak, S. (2012). Pollination biology of *Annona squamosa* L. (*Annonaceae*): evidence for pollination syndrome. *Scientia Horticulturae*, *144*, 212-217. DOI: https://doi.org/10.1016/j.scienta.2012.07.004

Koura, S., Hasegawa, K., Yamamoto, Y., & Yonemoto, Y. (2004). Fruit set and fruit growth of seedless cherimoya (*Annona cherimola* mill.) induced by GA_3 under greenhouse cultivation in Japan. *Acta Horticulturae*, *653*, 63-66. DOI: https://doi.org/10.17660/ActaHortic.2004.653.7

Lau, J. Y. Y., Pang, C.-C., Ramsden, L., & Saunders, R. M. K. (2017). Stigmatic exudate in the Annonaceae: pollinator reward, pollen germination medium or extragynoecial compitum? *Journal of Integrative Plant Biology*, *59*(12), 881-894. DOI: https://doi.org/10.1111/jipb.12598

Li, P.-S., Thomas, D. C., & Saunders, R. M. K. (2015). Phylogenetic reconstruction, morphological diversification and generic delimitation of disepalum (*Annonaceae*). *PLoS ONE*, *10*(12), 1-24. DOI: https://doi.org/10.1371/journal.pone.0143481

Li, X. J., Li, X., & Shu, H. (1998). Influences of boron, urea and plant growth regulators on the germination and growth of apple pollen. *Plant Physiology Communications*, *34*(2), 96-100.

Mahorkar, K. D., Naglot, U. M., Yadav, S. V., & Hake, P. D. (2020). Effect of plant growth regulators on yield and quality of custard apple (*Annona squamosa* L.). *International Journal of Current Microbiology and Applied Sciences*, *9*(3), 587-593. DOI: https://doi.org/10.20546/ijcmas.2020.903.069

Martin, C., Viruel, M. A., Lora, J., & Hormaza, J. I. (2019). Polyploidy in fruit tree crops of the genus *Annona* (Annonaceae). *Frontiers in Plant Science*, *10*(99), 1-14. DOI: https://doi.org/10.3389/fpls.2019.00099

Meade, C. V., & Parnell, J. A. N. (2018). A revised taxonomy for Uvaria (Annonaceae) in continental Asia. *Australian Systematic Botany*, *31*(4), 311-356. DOI: https://doi.org/10.1071/SB17051

Mota Filho, V. J. G., Pereira, M. C. T., Nietsche, S., Guimarães, J. F. R., Moreira, G. B. R., & Fernandes, T. P. (2012). Uso de fitorreguladores no desenvolvimento de frutos na atemoieira (*Annona cherimola* x *A. squamosa* cv. Gefner). *Revista Ceres*, *59*(5), 636-645. DOI: https://doi.org/10.1590/S0034-737X2012000500009

Moura, E. A., Chagas, P. C., Chagas, E. A., Oliveira, R. R., Siqueira, R. H., Taveira, D. L. L., … Grigio, M. L. (2019). Influence of seasonality in the production and quality of *Annona Squamosa* L. fruit of different sizes. *HortScience*, *54*(8), 1345-1350. DOI: https://doi.org/10.21273/HORTSCI14116-19

Moura, E. A., Chagas, P. C., Oliveira, R. R., Taveira, D. L. L., Grigio, M. L., & Araújo, W. F. (2021). Determination of the harvest time of sugar apples (*Annona Squamosa* L.) in function of carpel interspace. *Acta Scientiarum*. *Agronomy*, *43*(1), 1-10. DOI: https://doi.org/10.4025/actasciagron.v43i1.48732

Noble, C. V., Drew, R. M., & Slabaugh, J. D. (1996). *Soil survey of Dade County Area*, *Florida*. Washington, DC: Natural Resource Conservation Service, United States Department of Agriculture.

Peña, J. E., Nadel, H., Barbosa-Pereira, M., & Smith, D. (2002). Pollinators and pests of Annona species. In J. E. Peña, J. L. Sharp, & M. Wysoki (Eds.), *Tropical fruit pests and pollinators: biology*, *economic importance*, *natural enemies and control* (p. 197-221). Wallingford, UK: CABI.

Pereira, M. C. T., Crane, J. H., Montas, W., Nietsche, S., & Vendrame, W. A. (2014a). Effects of storage length and flowering stage of pollen influence its viability, fruit set and fruit quality in 'Red' and 'Lessard Thai' sugar apple (*Annona squamosa*) and 'Gefner' atemoya (*A. cherimola* × *A. squamosa*). *Scientia Horticulturae*, *178*, 55-60. DOI: https://doi.org/10.1016/j.scienta.2014.08.004

Pereira, M. C. T., Nietsche, S., Crane, J. H., Montas, W., Siqueira, C. L., & Rocha, J. S. (2019). Gibberellic acid combined with hand pollination increases 'Red' and 'Lessard Thai' sugar apple fruit quality and produced parthenocarpic 'Gefner' atemoya fruits. *Ciência Rural*, *49*(9), 1-5. DOI: https://doi.org/10.1590/0103-8478cr20180353

Pereira, M. C. T., Santos, R. K. A., Nietsche, S., Mizobutsi, G. P., & Santos, E. F. (2014b). Doses de ácido giberélico na frutificação efetiva e qualidade de frutos de atemoieira 'Gefner'. *Revista Brasileira de Fruticultura*, *36*(spe1), 184-191. DOI: https://doi.org/10.1590/S0100-29452014000500022

R Core Team. (2020). *R: A language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing.

Ramezani, S., & Shekafandeh, A. (2009). Roles of gibberellic acid and zinc sulphate in increasing size and weight of olive fruit. *African Journal of Biotechnology*, *8*(24), 6791-6794. DOI: https://doi.org/10.4314/ajb.v8i24.68670

Reig, C., Mesejo, C., Martínez-Fuentes, A., & Agustí, M. (2014). Naphthaleneacetic acid impairs ovule fertilization and early embryo development in loquat (*Eriobotrya japonica* Lindl.). *Scientia Horticulturae*, *165*, 246–251. DOI: https://doi.org/10.1016/j.scienta.2013.11.030

Santos, R. C., Pereira, M. C. T., Mendes, D. S., Sobral, R. R. S., Nietsche, S., Mizobutsi, G. P., & Santos, B. H. C. (2016). Gibberellic acid induces parthenocarpy and increases fruit size in the 'Gefner' custard apple (*Annona cherimola* x *Annona squamosa*). *Australian Journal of Crop Science*, *10*(3), 314–321. DOI: https://doi.org/10.21475/ajcs.2016.10.03.p6911

Santos, R. C., Nietsche, S., Pereira, M. C. T., Ribeiro, L. M., Mercadante-Simões, M. O., & Santos, B. H. C. (2019). Atemoya fruit development and cytological aspects of GA 3-induced growth and parthenocarpy. *Protoplasma*, *256*(5), 1345-1360. DOI: https://doi.org/10.1007/s00709-019-01382-2

Verotti, T. P., Oliveira, C. G., Souza Parreiras, N., Gonçalves, F. C. M., Corrêa, C. V., Ferreira, G., … Boaro, C. S. F. (2019). Vegetal regulators increase the quality of atemoya fruits and recover the photosynthetic metabolism of stressed plants. *Acta Physiologiae Plantarum*, *41*(165), 1-11. DOI: https://doi.org/10.1007/s11738-019-2960-4

Wang, X.-F., Armbruster, W. S., & Huang, S.-Q. (2012). Extra-gynoecial pollen-tube growth in apocarpous angiosperms is phylogenetically widespread and probably adaptive. *New Phytologist*, *193*(1), 253-260. DOI: https://doi.org/10.1111/j.1469-8137.2011.03912.x.

Yang, Q., Wang, Y., Fu, Y., Deng, Q., & Tao, L. (2012). Effects of biological factors on fruit and seed set inloquat (*Eriobotrya japonica* Lindl.). *African Journal of Agricultural Research*, *7*(38), 5303-5311. DOI: https://doi.org/10.5897/AJAR12.1274.