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Determining the preliminary performance of selected Annonaceae cultivars in subtropical conditions

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Abstract

Plants phenology is extremely important tool for crop management, crop manipulation, and marketing. The objective of this research was to investigate the phenology and fruit quality of the several Annonaceae species under subtropical conditions of Homestead, Florida, USA. The study was carried out in an experimental sugar apple (*Annona squamosa* L.), orchard with cultivars: 'LeahReese', 'Lessard Thai' and 'Red' and the atemoya 'Gefner' (*A. cherimola* x *A. squamosa* L). Ten uniformly vigorous and healthy plants of each cultivar were selected. The fruit harvest period was similar for all the cultivars, except for sugar-apple 'LeahReese', which had the shortest harvest period of only 41 days. Multivariate analysis showed that 'Lessard Thai' and 'Red' sugar apple cultivars had smaller sizes and lower chemical quality. Cultivar "LeahResse" had the highest fruits, in addition, a strong correlation with the atemoya hybrid "Gefner". The sugar apples 'Lessard Thai' and 'Red' had similar phenology and had a 166-day pruning to harvest cycle. In contrast, 'LeahReese' sugar apple and 'Gefner' atemoya had significantly longer cycles of 182 and 196 days, respectively. The sugar apple 'LeahReese' had the best physical characteristics of the cultivars investigated, while the atemoya 'Gefner' had the best physiochemical characteristics and the longest shelf life.

Keywords: *A. cherimola* x *A. squamosa;* developmental stages; flowering stage; phenological study; seasonal variation

Introduction

Annonaceae (annona) are considered easily adaptable, being grown in regions of tropical and subtropical climate with excellent productivity. However, despite their easy adaptation to climatic conditions, species and cultivars show variations in their phenological phases due to climate differences (Moura et al., 2020; Alves Rodrigues et al. 2018; Liu et al. 2015; Mendes et al. 2017; Moura et al., 2019a; Pereira et al., 2014a). These seasonal variations influence each species, genotype, and cultivar even among orchards of differing production regions e.g., Florida in contrast to Ecuador (Camargo et al., 2011; Moura et al., 2020, 2019b; Olesen & Muldoon 2012; Oliveira et al., 2018; Rivero et al., 2017).

There are a number of sugar apple cultivars in south Florida including 'Thai Lessard' (a green type), 'Purple' or 'Red', 'Kampong Mauve' (red-purple types), and a seedless type known under various names, 'Cuban Seedless' and 'Brazilian Seedless'. Atemoya is an interspecific hybrid of sugar-apple (*Annona squamosa* L.) and cherimoya (*Annona cherimola* Mill.). The fruit combines qualities of the two species including the sweet taste of cherimoya, much appreciated by consumers and adaptation to slightly cooler regions than sugar apple.

Both sugar-apples and atemoyas have excellent physicochemical qualities, with changes in chemical composition during fruit ripening (Moura et al., 2021, 2019b; Santos et al., 2016), which makes their taste and aroma more pleasant. The value of annona varies according to the internal and external quality of the fruits. Fruit quality characteristics such as taste and odor, texture, nutritional value, size, symmetry, mass, color, firmness, sweetness, acidity, physical and physiological defects among others are influenced by the long- and short-term environmental conditions of the orchard (Moura et al. 2021; Moura et

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al. 2019b), therefore, the phenological knowledge of cultivars is extremely important.

The organoleptic characteristics of fruits have a defined genetic range and gene expression is influenced by climatic conditions and orchard management (Moura et al., 2021, 2019b). The variation in the physical characteristics of fruits of annona species is also related to pollen source and load (Lau et al., 2017), pollen viability (Araújo et al., 2021; Pereira et al., 2014a) and the methodology and timing of pollination (Alves Rodrigues et al., 2018; Mota Filho et al., 2012; Pereira et al., 2014b).

The phenological parameters for some Annonaceae cultivars is not well known. Therefore, it is important to determine the phenological behavior and physical and physicochemical characteristics of the cultivars within a production area before being marketed. The objective of the present study was to determine the phenology and fruit quality of selected sugar apple cultivars and 'Gefner' atemoya under the subtropical conditions of Homestead, Florida, USA.

Material and Methods

Site Characterization

The experiment was carried out at the University of Florida, Tropical Research and Education Center (UF/TREC, Homestead, Florida, U.S.A.) experimental sugar apple orchard (*Annona squamosa* L.), using 'LeahReese', 'Lessard Thai' and 'Red' and the atemoya 'Gefner' orchard (*Annona cherimola* Mill x *Annona squamosa* L.). UF/TREC is located at latitude: 25° 30 '40.809' 'N by longitude: 80° 30' 3.983 '' W and at an altitude of 3.8 m. The region has a marine subtropical climate with an average annual rainfall of 1,490 mm [UF/TREC Florida Automated Weather Network (http://fawn.ifas.ufl.edu/] with the majority of rainfall (70%) occurring from May to early November (Ali et al. 2000).

The meteorological data collected during the experimental period were rainfall, maximum, average, and minimum temperature, and relative humidity (Figure 1).

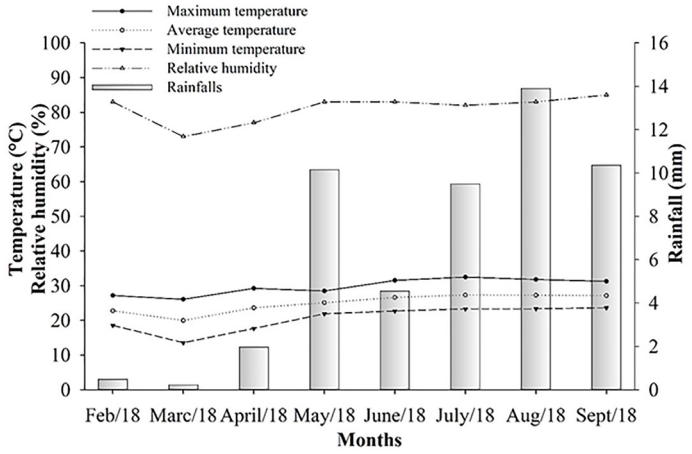

Figure 1. Maximum (°C), average (°C) and minimum (°C) temperatures, rainfall (mm), and relative humidity (%) for the months of the Homestead - Florida-USA, 2018.

The native soil is classified as a loamy-skeletal, carbonatic, hyperthermic Lithi Udorthent which is modified by scarification and trenching the oolitic bedrock to facilitate tree establishment and stability (Noble et al., 1996). Potential root depth in-row trenches were 45 cm deep by 40 cm wide and 15 cm deep among tree rows. The soil is well drained, with a low water holding and cation exchange capacity and high pH (7.2-8.5).

The experiment commenced during the second half of February 2018. Ten plants of each cultivar were selected for uniformity, vigor, and health. The plants were grown in a 6 m x 4 m spacing. The 'Lessard Thai' were planted in 2000, 'Red' in 1997, 'LeahReese' in 2012, and the atemoya 'Gefner' in 1999.

Cultural practices

The trees were mechanically and manually pruned in the second half of February 2018. During the growing season, April through August, the orchard was irrigated twice per week to apply about 25 mm water per application, if rainfall did not exceed 1 cm/week. The weeds and grass in the row-middles was controlled using a mechanical brush-cutter every two months and chemical control was used to control in-row weeds. Fertilization was performed according to soil analysis and crop requirement after pruning.

Variables analyzed

The plants were evaluated every 4 days, from

pruning until the harvest of the fruits (physiological maturation), to identify precisely each of the phenological stages: (1) emergence of vegetative buds and the beginning of the shoot extension when 50% of the plant vegetative buds started to sprout and; (2) commencement of flowering and flowering duration, flowering start dates were recorded, when 5% of flower buds were emerged on the plants, and when flowering plants no longer presented new flowers. Based on the flowering data, the flowering duration was obtained by calculating the interval in days between the beginning and the end of flowering. The harvest period i.e., start and end dates were recorded. Harvesting began when the first fruits reached physiological maturity and was considered ended when the plants no longer had fruits to be harvested. Based on the harvest data, the harvest period was calculated by the interval in days between the beginning and the end of the harvest.

At the time of full bloom, manual pollination was carried out between 6 am and 11 am with pollen collected in the morning from flowers at the male stage of opening. The pollen grains collected were placed in Eppendorf-type flasks. Then, with using a brush number 6, the pollen was deposited on the stigma of the flowers in the female stage (anthesis), for each cultivar. The manual pollination of 'Lessard Thai' was held on May 10th, 'Red' on May 9th, 'LeahReese' on May 7th and ateymoa 'Gefner' on May 7th, 2018.

To evaluate the quality of production of each cultivar, fruits were harvested at physiological maturity. The fruits of each cultivar were identified, protected in paper bags, packaged in plastic boxes and taken to the UF/TREC Horticulture. Fruit were evaluated for fresh mass (g), length (mm), and diameter (mm). Afterwards, the fruits were kept in the laboratory at a constant temperature of 25 °C until ripe.

When fruits were ripe, a digital scale (Model TR-6161, Denver Instruments, Arvada, CO) was used to record fresh fruit mass, fresh peel mass plus peduncle, and fresh pulp. Pulp yield was calculated by subtracting the fruit mass from the peel plus peduncle and seed mass and the results expressed as a percentage. The number of seeds per fruit were recorded. Soluble solids were determined with a digital refractometer (Model PAL-1BLT/A+W, Atago Co. Ltd., Tokyo, Japan) and the values expressed as a percentage (AOAC 2012). Pulp pH (hydrogen potential) and titratable acidity were determined by titration with sodium hydroxide solution (0.1N) using the Compact Titrator G20S (Mettler Toledo®, Columbus, OH) and the results expressed in grams of 100 g^{-1} pulp citric acid. The acid/soluble solids ratio was then calculated.

Experimental Design

For the phenological study, the experimental design was randomized blocks with 4 treatments (cultivars) and 10 replications. For the evaluation fruit quality, a completely randomized design with 12 fruits of each treatment (cultivars), with 4 repetitions and 3 fruits per repetition.

Statistical Analysis

Analyses of variance (ANOVA) were performed by the F ($p<0.01$). Post hoc mean comparisons were carried out according to the Tukey–Kramer test ($p <0.05$). Data are expressed as mean ± standard deviation. The significant variables were subjected to multivariate analysis. Multivariate data analysis was performed by principal component analysis (PC). The analyzes were performed using the computer program R (R Core Team, 2018).

Results

Phenological Behavior

Annual pruning was used to control tree size and to synchronize the growth stages of each cultivar. The pruning removed about 1 m of the top of the canopy and 0.75 m of the side canopy was performed on the same day for all cultivars (Table 1).

Table 1. Dates observed for each phenological stage during the 2018 productive cycle of 'Lessard Thai', 'Red', 'LeahReese' and 'Gefner' in Homestead, Florida-USA, 2018.

Phenological stage	Cultivars			
	'Lessard Thai' ugar apple	'Red' sugar apple	'LeahReese' sugar apple	'Gefner' atemoya
Production pruning	Feb 16/18	Feb 16/18	Feb 16/18	Feb 16/18
Beginning of bud swelling	March 02/18	March 02/18	March 09/18	March 02/18
Beginning of flowering	March 30/18	March 30/18	April 03/18	March 30/18
End of flowering	June 25/18	June 25/18	June 25/18	June 25/18
Beginning of harvest	Aug 01/18	Aug 01/18	Aug 17/18	Sept 01/18
End of harvest	Oct 01/18	Oct 01/18	Sept 28/18	Nov 03/18
Total days of the cycle (pruning to fruit harvest)	166	166	182	196
Total days of the cycle (hand pollination to fruit harvest)	95	93	103	125

The sugar-apple cultivars 'Lessard Thai' and 'Red' behaved similarly for all phenological stages, with a pruning to harvest cycle of 166 days. However, for the sugar-apple cultivar 'LeahReese' and atemoya 'Gefner', the pruning to harvesting cycle was longer, 182 and 196 days, respectively. The interval in days from manual pollination of flowers to fruit harvest was 95 days for 'Lessard Thai', 93 days for 'Red', 103 days for 'LeahReese' and 125 days for 'Gefner'.

Physical characteristics of fruits

For the fruit physical characteristics, 'LeahReese' was significantly superior for most variables compared to the other cultivars, except for fruit length and fruit mass, which did not differ statistically from the atemoya cultivar 'Gefner' (Table 2). The percentage of 'Gefner' pulp' was significantly higher than all the sugar apple cultivars and 'LeahReese' had more pulp than the other sugar apples (Table 2).

Table 2. Physical characteristics of fruits of cultivars of 'Lessard Thai', 'Red', 'LeahReese' and 'Gefner' in Homestead, Florida-USA, 2018.

Cultivars	'Lessard Thai' sugar apple	'Red' sugar apple	'Leah 'Reese' sugar apple	'Gefner' Atemoya	C.V
Length (mm)	87.93±3.68 b	89.90±5.96 b	120.25±2.96 a	121.13±12,91 a	7.57
Diameter (mm)	89.70±2.55 c	100.63±2.69 b	118.36±2.83 a	105.21±3.01 b	2.69
Fruit mass (g)	309.27±21.46 c	400.10±46.39 b	627.27±21.95 a	591.28±15.61 a	5.99
Peel + stem mass (g)	161.52±11.45 b	206.36±24.94 b	291.14±23.07 a	112.05±2.11 c	9.32
Seed mass (g)	17.16±0.58 c	20.93±0.97 bc	32.83±2.94 a	24.01±1.92 b	7.77
Number of Seeds	40.33±2.90 b	45.33±2.30 b	74.11±8.88 a	51.44±5.79 b	10.64
Pulp (%)	41.92±0.89 c	42.78±1.80 c	48.76±1.81 b	76.98±0.78 a	2.68

Means followed by the same letter in the row do not differ by the Tukey–Kramer test ($p<0,05$). Data are expressed as mean±standard deviation ($n=144$).

These results suggest the great productive potential of the new sugar-apple cultivar 'LeahReese' compared to all others evaluated. The better performance of 'Gefner' atemoya for pulp yield can be explained by the fact that atemoya, in general, have thinner peel when compared to sugar apples (Table 2).

Chemical characteristics

'Gefner' atemoya had significantly greater pulp SS (Brix), pH, lower acidity and greater shelf-life compared to the sugar apples (Table 3).

Table 3. Physicochemical characteristics of fruits of cultivars of 'Lessard Thai', 'Red', 'LeahReese' and 'Gefner' in Homestead, Florida-USA, 2018.

Cultivars	'Lessard Thai' sugar apple	'Red' sugar apple	'Leah 'Reese' sugar apple	'Gefner' Atemoya	C.V
SS (°Brix)	22.80±0.65 c	23.54±0.25 bc	25.55±1.10 b	31.37±1.33 a	3.65
pH	4.87±0.04 b	4.84±0.07 b	4.83±0.06 b	4.57±0.01 a	1.13
Acidity (g 100 g polpa^{-1})	0.21±0.07 b	0.22±0.04 b	0.23±0.03 b	0.39±0.01 a	11.43
Ratio	105.25±4.11 a	115.05±23.04 a	117.16±20.34 a	80.21±2.96 a	14.92
Shelf Life	4,67±1 b	3.00±0.58 c	4.00±0.01 bc	7.00±0.01 a	12.37

Means followed by the same letter in the row do not differ by the Tukey–Kramer test ($p<0,05$). Data are expressed as mean±standard deviation ($n=144$).

'LeahReese' had significantly greater SS than 'Lessard Thai' but similar to 'Red' sugar apple. Pulp pH and acidity were similar among all sugar apples. Interestingly, there was no significant difference in the acid/SS ratio among any cultivars tested. Shelf-life of 'Gefner' atemoya was significantly longer than all sugar apples with 'Lessard Thai' having a significantly longer shelf-life that 'Red' and 'LeahReese' (Table 3).

Principal Component Analysis

To better understand the behavior of physical and physicochemical characteristics of annona cultivars investigated, a multivariate analysis was performed by principal component analysis (PC) using all the variables (Figure 2). The variance explained by the two main components was 91.20% effective in discriminating the principal components.

PC1 and PC2 explained 54.70 and 36.50% of the data variability, respectively. PC1 correlated positively with fruit length, mass, % pulp, ° Brix, acidity and Shelf Life (Vprat), and negatively correlated with pH and acid/SS ratio. 'Gefner' atemoya presented excellent levels of °Brix, acidity and had longer fruit shelf life, variables that showed an inverse correlation with pH and acid/SS ratio (Figure 2).

PC2 correlated strongly negatively with diameter, peel mass, seed mass and number of seeds (N seed). The variables Vprat, % pulp, acidity, and °Brix were correlated with 'Gefner' and explained 63.61% of the data variability in PC1. The variables length, fruit mass, diameter, seed

mass and N seed, peel mass, acid/SS ratio and pH had the highest correlation with the sugar apple cultivar 'LeahReese', and explained 68.55% of the data variability in PC2. The cultivars with the lowest direct correlation with the variables were 'Lessard Thai', explaining 19.22% in PC1 and 20.50% in PC2 and 'Red', explaining 16.88% in PC1, respectively.

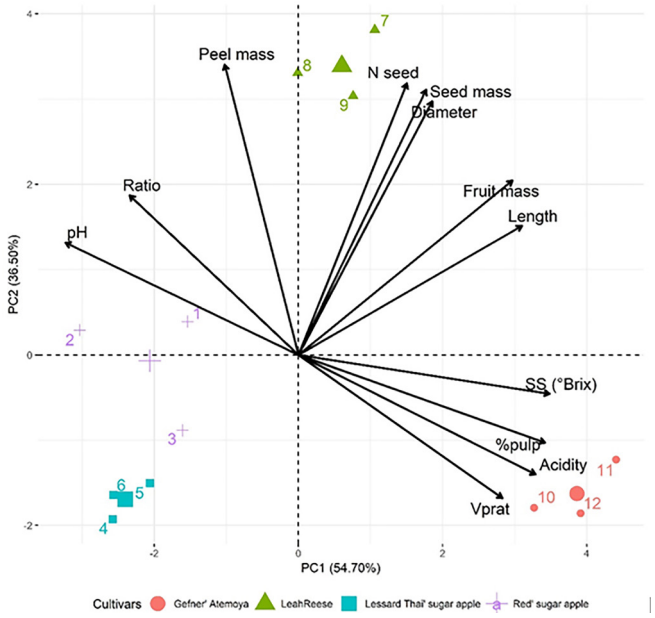

Figure 2. Principal component analysis (CP) performed with Annonaceae cultivars: 'Lessard Thai' sugar apple; 'Red' sugar apple; 'LeahReese' sugar apple and the hybrid 'Gefner' atemoya on the physical and physicochemical characteristics of the fruits. Biplot (load variables and sample scores): open diamond symbols correspond to physical and physicochemical characteristics; open circle symbols correspond to Annonaceae cultivars ($n = 144$).

Discussion

Phenological Behavior

Of the sugar apples investigated the phenological stages were longer for 'LeahReese', which delayed the onset of shoots by 7 days and the flowering by 4 days in relation to the other cultivars. However, the end of flowering occurred at the same time for all sugar apple cultivars evaluated (Table 1), while the end of flowering occurred at the same time for all cultivars. Differences were observed for the beginning of the fruit harvest, where the cultivars 'LeahReese' and 'Gefner' were delayed by 16 and 30 days compared to 'Lessard Thai' and 'Red', respectively. Similar responses to those found in the present study were found by (Pereira et al. 2014a), who obtained artificial pollination intervals at 97 days for 'Lessard Thai', 98 days for 'Red' and 115 days for 'Gefner' and with *A. squamosa* L, respectively (Moura et al. 2019a) (Table 1).

The fruit harvest period was similar for the cultivars (± 60 days), except for 'LeahReese', which had a shorter harvest period than the others, lasting only 41 days. This harvesting period in Florida varies depending on the season, as fruits generally do not ripen at the same time and harvesting can extend from 3 to 6 months if the season is from mid-summer to late winter, which can be further aggravated by cool temperatures and/or frost (Crane et al., 2016).

This variation in phenological stages is closely related to climate conditions during the tree and fruit developmental period (Moura et al., 2019b). Plant species and cultivars within a species may respond differently to the climatic conditions experienced during the production period (Matsuda & Higuchi 2012). Thus, rapid temperature increases in the period of growth and reproductive development may lead to early or delayed flowering. This has been documented to occur in sugar apple grown under semiarid and tropical savanna conditions (Alves Rodrigues et al., 2018; Moura et al., 2020, 2019a, b).

The vegetative and reproductive behavior of annonas is managed by several phenological and physiological attributes, such as leaf morphology, inherent plant vigor, timing of bud emergence, transpiration rate and photosynthetic rate (Liu et al., 2015). These substantial seasonal variations in growth, development, and physiological attributes may vary for each species

and cultivar within a species (or hybrid), as reported for atemoya (Olesen & Muldoon 2012) and sugar-apple (Alves Rodrigues et al. 2018; Liu et al. 2015; Moura et al. 2019b). Therefore, the duration of each phenological stage of the species will differ depending on some factors, such as genotype, edaphoclimatic conditions and crop management (Moura et al., 2020). The identification of the phenological phases for each cultivar and their duration may allow for manipulating these crops during favorable and unfavorable periods of the vegetative and reproductive cycles (Moura et al., 2020, 2019a).

All cultivars investigated had superior fruit size to those observed in the literature. Mota Filho et al. (2012), reported 'Gefner' atemoya fruit length to be 92 mm in length and 84 mm in diameter. In one study, Pereira et al. (2014b) reported 94.6 mm in length and 85.9 mm in diameter for hand pollinated 'Gefner'. In another study, Pereira et al. (2014a) reported 104 mm length and 90 mm in diameter for 'Gefner', 88 mm length and 87 mm diameter for 'Red' and 88 mm length and 84 mm diameter for 'Lessard Thai'.

In the present study, manual pollination of flowers was performed, and the results obtained confirm the benefits of this management practice. The use of artificial pollination in commercial Annonaceae orchards significantly increases the yield of these crops (Araújo et al., 2021). Artificial pollination increases fruit size, results in symmetrical fruit shape and improves various other physical and chemical characteristics of the fruit (Mota Filho et al., 2012; Pereira et al. 2014b, a).

Documenting the phenological phases of individual cultivars is basic for development of manipulative procedures (e.g., pruning, plant growth regulator applications) that may induce early or late season production and scheduling of various production practices and labor (e.g., pollination, fertilization).

Physical and chemical characteristics

The soluble solids content of atemoya 'Gefner' was superior to those cited in the literature (Pereira et al. 2014b). In contrast, the sugar apple cultivars investigated had relatively good soluble solids contents, however, slightly below those observed in the literature (Moura et al., 2021, 2019b), especially when compared to fruits from Brazil. This may be attributed to higher and more consistent high ambient temperatures in the Brazilian annona region than south Florida. However, these cultivars still had soluble solids content within the acceptable ranges for the market.

Although the atemoya 'Gefner' had the highest average pH all cultivars are within the ranges observed in the literature (Table 3). In general, the lower the fruit pH, the greater the potential postharvest protection from decay and the longer the shelf life. This was observed for the atemoya hybrid cultivar 'Gefner', which obtained 7 days of life after harvest (Table 3).

The titratable acidity is directly involved with the organoleptic quality of the fruits, influencing its taste and odor, being therefore, a key characteristic that defines fruit quality. Sugar apple cultivars had titratable acidity values similar to those observed in the literature (Moura et al., 2021) but higher than those observed by Pereira et al. 2014b, averages of 0.16 g of citric acid 100 g^{-1} pulp. The low titratable acidity presented in the fruits of sugar-apple cultivars must have occurred due to their rapid maturation, not producing enough organic acids to increase the titratable acidity. The titratable acidity in some species decreases during ripening; in others, acid accumulation occurs during ripening. This effect is associated with reduced enzymatic activity (malate oxidase) (Vanlerberghe et al., 2020) since acid accumulation during ripening is common in Annonaceae and has been observed in several species (Moura et al., 2021).

The low titratable acidity of sugar apple cultivars can be explained by the shorter shelf life compared to the 'Gefner' atemoya hybrid cultivar (Table 3). Organic acids influence sweetness and also have relevance when used for industry, as acidity can serve as a preservative and provide longer shelf life (Aroucha et al., 2010).

The atemoya cultivar 'Gefner' had 7 days shelf life compared to about 3 to 4 days for the sugar apple cultivars investigated (Table 3). Short shelf-life limits the potential distance fruit can be marketed to and the short shelf-life of sugar applies reduces their economic potential.

Conclusions

The sugar apples 'Lessard Thai' and 'Red' had similar phenology and had a 166-day pruning to harvest cycle. In contrast, 'LeahReese' sugar apple and 'Gefner' atemoya had significantly longer cycles of 182 and 196 days, respectively. The sugar apple 'LeahReese' had the best physical characteristics of the cultivars investigated, while the atemoya 'Gefner' had the best physiochemical characteristics and the longest shelf life.

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