Acta Scientiarum
http://periodicos.uem.br/ojs
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v43i1.51013

Flower stages, germination and viability of pollen grains of *Annona squamosa* L. in tropical conditions

Digelma Camila Barros Araújo[1], Pollyana Cardoso Chagas[1], Edvan Alves Chagas[2], Elias Ariel Moura[3*], Railin Rodrigues de Oliveira[1], Daniel Lucas Taveira[1], Maria Isabel Ribeiro[1] and Maria Luiza Grigio[1]

[1]Universidade Federal de Roraima, Campus Cauamé, BR-174, Km 12, Monte Cristo, Roraima, Brazil. [2]Empresa Brasileira de Pesquisa Agropecuária, Boa Vista, Roraima, Brazil. [3]Universidade Federal Rural do Semiárido, Mossoró, Rio Grande do Norte, Brazil. *Author for correspondence. E-mail: eliasariel90@gmail.com

ABSTRACT. The success in the application of artificial pollination techniques, aspects of floral biology should receive special attention, especially regarding the viability studies of pollen grains. In this sense, two experiments were carried out, aiming to determine the floral stages: floral opening (anthesis), female, male and senescence stages of *A. squamosa* L flowers under tropical climate conditions, and to evaluate the germination and viability of pollen grains submitted to different storage conditions. In the first experiment, observations and data collection began when flowers were still closed. Readings were taken every two hours for 46 hours until all the flowers reached the senescence stage. For the second, the experimental design was completely randomized, in a 2 x 7 factorial scheme, with 2 storage conditions (environment at 27 ± 2°C and refrigerated at 5 ± 2°C) and 7 pollen grain conditioning times (0, 4, 8, 12, 16, 20 and 24 hours), with 4 repetitions for the analysis of pollen grain germination and 3 repetitions for the analysis of pollen grain viability, and each repetition consisted of one blade. The variables evaluated were percentage of viable and non-viable pollen grains and percentage of germinated and non-germinated pollen grains. For tropical climatic conditions, the flower anthesis (female stage) of the sugar-apple begins at 00:00 h, extending until 12:00h on the same day. The flowers of the tree reach a functionally staminate stage (male stage) from 6 a.m. the next day. Pollen grains stored at room conditions (27 ± 2°C) remain viable for up to 24 hours after collection, showing that storage in a cold environment at low temperatures (5 ± 2°C) is not necessary. Seed pollen grains have a germination percentage of 51.25% when stored under ambient conditions (27 ± 2°C) for up to 4 hours after collection.

Keywords: floral biology; breeding; female stage; male stage; senescence.

Received on November 16, 2019.
Accepted on March 11, 2020.

Introduction

The sugar apple tree (*Annona squamosa* L.) is species is native to tropical America, particularly to the Antilles, and can be cultivated in tropical and subtropical areas (Liu et al., 2015). Is one of the most important species of the genus *Annona*, due to the commercial value of its fruits and to the much-appreciated taste by consumers (Zucareli, Ferreira, Silvério, & Amaro, 2008). In addition to dietary properties, Annonaceae have medicinal, pharmaceutical (Madhu, Brainard, Raj, Swapn, & Rao, 2012; Manvi, Nanjwade, & Shing, 2011) and nutritional properties such as vitamins A, B, C, E, K1, antioxidants, polyunsaturated fatty acids and essential minerals, in addition to its pleasant aroma and flavor (Liu et al., 2015; Liu, Yuan, & Jing, 2013), as well as its potential as insecticide (Seffrin, Shikano, Akhtar, & Isman, 2010).

The *A. squamosa* L is strongly affected by climatic conditions and depending on the seasonal variations of each biome, an advance or delay in the crop cycle may occur, affecting the viability of the floral set and the quantity and quality of fruits. Second, the problem of inefficient pollination is due to high temperatures (30°C) and low relative humidity (30%), while low temperature (25°C) and high relative humidity (80%) enable efficient pollination (Khalate, Supe, & Doke, 2018).

Rodrigues et al. (2016) studying different temperatures for the in vitro pollen grain germination of *A. squamosa*, found that the temperature of 25 ± 1°C provided the best in vitro germination, with 48.13%, and as the temperature increased, germination percentage decreased significantly. George and Nissen (1988) cite that high temperatures adversely affect flower bud production, suggesting that tropical conditions are not conducive to high sugar-apple yields.

Therefore, it is observed that the greater the climatic factors at the time of floral development and anthesis (high temperatures, low relative humidity and high vapor pressure deficit), the greater the likelihood that flowers will reach floral abscission as well as stigma desiccation before floral dehiscence, noting that the flower exhibits protogynous dichogamy. Thus, strategies that allow the extension of the floral stage, as the flower's receptive period and the viability of pollen grains, to obtain satisfactory *A. squamosa* production in regions with tropical and subtropical climatic conditions are necessary.

Pollen viability is one of the factors that have a direct influence on fertilization success. It is recommended to test pollen viability before use (Einhardt, Correa, & Raseira, 2006). The success of in vitro germination depends on several factors such as plant species, plant nutritional status, time of year and time of collection, photoperiod, air temperature, collection method, incubation period and presence of micro and macronutrients in the culture medium (Soares et al., 2008), as well as adjustments of the culture medium composition for each species (Chagas, Pio, Chagas, Pasqual, & Bettiol Neto, 2010; Sinimbú Neto, Martins, & Barbosa, 2011).

In vitro and in vivo pollen germination allows the analysis of pollen tube emission capacity and a correlation of this rate with pollen grain viability (Ferreira et al., 2007). However, the determination of pollen grain viability by dyes is a common practice in cytogenetics. Colorimetric methods use specific chemical dyes that react with cellular components present in mature pollen grains, such as lugol and acetic carmine. Some dyes are more commonly used than others, such as Tetrazolium salt, Lugol's solution, Alexander's solution and Acetic carmine (Einhardt et al., 2006).

Studies on pollen grain are the basis for understanding reproductive biology and are important for breeding, conservation of plant genetic resources and hybrid seed production (Nascimento, Torres, & Lima, 2003) However, despite existing research nitiated a few decades ago, information is scarce for tropical climate conditions in the world, especially in the Northern Amazon, Brazil.

Therefore, the aim of this study was to determine the flowering stages, and to evaluate the germination and viability of short-lived pollen grains subjected to different storage conditions, under the tropical climate conditions, Northern Amazon of Brazil.

Material and methods

The study was conducted at *A. squamosa* L. commercial orchard in Cantá, Roraima state, Brazil (Latitude: 2° 36' 30' 'North, Longitude: 60° 35' 46'' West). The plants were 8 years old, implanted at a 4 x 5 m spacing. The climate of the experimental region is tropical rainy, type Aw according to the Köppen classification (Alvares, Stape, Sentelhas, Moraes Gonçalves, Sparovek, 2013), with an average temperature of 27.4°C and 90 m altitude, with a minimum annual precipitation of 944.7 mm, totaling 1,678.6 mm per year and relative humidity 70% (Araújo, Andrade Junior, Medeiros, & Sampaio, 2001).

During the experimental period they were measured at average temperatures and relative humidity, as shown in Figure 1.

For the experiment, 100 completely closed flower buds were selected, in a completely randomized design with 4 replications and under homogeneous conditions, which were marked with fluorescent labels two hours before the beginning of the observation. Observation and data collection began at the time when the flowers were still closed at 4 pm. Thereafter, the readings were taken every two hours for 46 hours when all the flowers reached the senescence stage.

The evaluations were performed in February 2015. Data collected during this period consisted of annotations of the morphological changes of each flower during the observation period. Thus it was possible to accurately determine the period when the flowers reached the feminine stage, masculine stage and senescence.

The second experiment was carried out at Embrapa Roraima's Tissue Culture and Plant Pathology Laboratory. In order to study the viability and in vitro germination of pollen grains, 100 randomly selected flowers were collected at 6 p.m., in homogeneous conditions at the beginning of the female stage (petals slightly apart). The flowers were taken to the Tissue Culture laboratory in single-layer plastic trays at room temperature (around 27 ± 2°C). The next day, at 5:30 a.m., when the flowers were in the male stage, the pollen grains were separated, divided into equal parts and placed in plastic containers, closed and identified as ‘pollen refrigerated’ and ‘ambient pollen’.

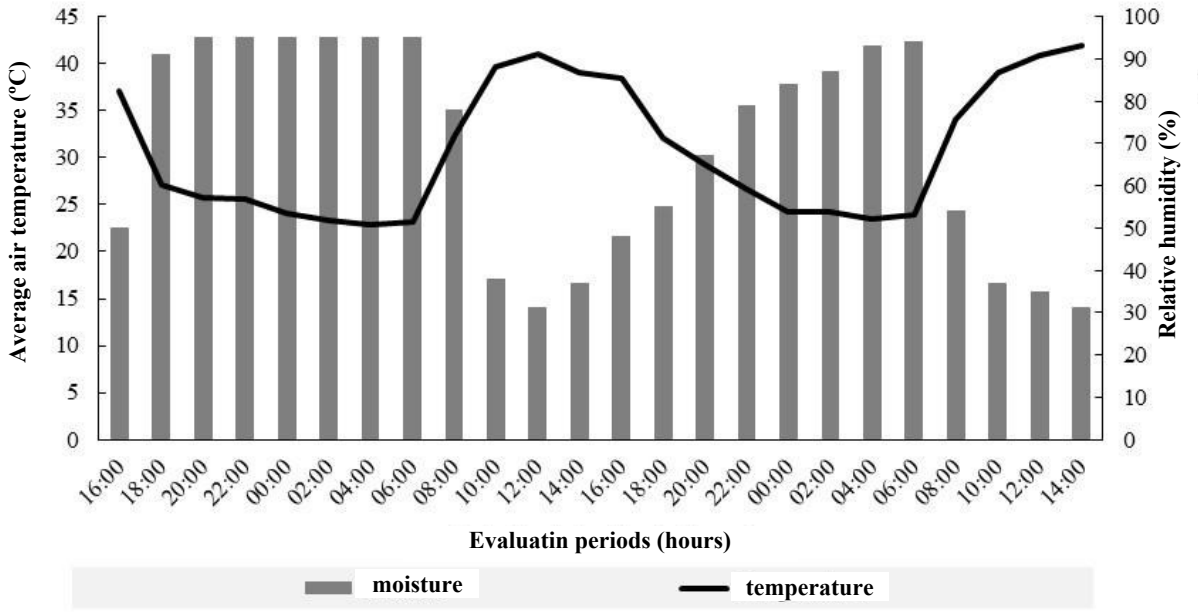

Figure 1. Average temperature (°C) and relative humidity (%) for the evaluation periods of the experiment. Cantá, Roraima state, Brazil, 2015.

Thereafter, the bottle labeled 'ambient pollen' was stored under ambient conditions at around 27 ± 2°C, while 'refrigerated pollen' was kept refrigerated at a temperature of 5 ± 2°C. Immediately after separation and identification of conservation treatments, in vitro germination and viability tests were performed, and the first evaluation was performed (0 hours of conditioning), at 8 a.m., shortly after pollen collection. The other evaluations were performed every 4 hours after the pollen grains were packed at 4, 8, 12, 16, 20 and 24 hours for both pollen subjected to room temperature and for pollen stored under refrigeration.

In order to evaluate the viability of pollen grains, the technique described by Linsley & Cazier (1963) was adapted, including staining of pollen grains arranged on a glass slide with 1% cotton blue.

In the in vitro germination test, the culture medium composed of 0,076 mg L^{-1} of Ca (NO_3) 2; 0,015 mg L^{-1} de $MgSO_4$; 0,017 mg L^{-1} of KNO_3; 0,029 mg L^{-1} of $H_3 BO_3$; 5 g of Agar (Brewbaker & Kwack, 1963). The medium was distributed in petri dishes. To the culture medium 10% sucrose was added according to the methodology used by (Loguercio, 2002). After pollen inoculation, the petri dish was left under room temperature conditions (27°C), as recommended by (Lorenzon & Almeida, 1997).

The inoculated plate was surface washed with 15 drops of pure water to detach the pollen grains. Shortly thereafter, a drop of the pollen containing solution and a drop of lactophenol cotton blue were mixed in a slide. Then 1 drop of this solution was pipetted into the Neubauer chamber and visualized under the microscope. Four observation slides were mounted for each inoculated plate, facilitating visualization for the germinated pollen count.

The method used to test pollen viability was an adaptation of the tetrazolium technique (Dafni, 1993). Pollen samples were collected every 4 hours in the different storage treatments for slide preparation with 2, 3, 5 triphenyl tetrazolium chloride (TTC) at a concentration of 1%. Pollen grains were distributed on 3 slides per treatment and one drop of dye added to each slide. The slides were left for four hours to check staining. Slides with 80% red stained pollen were considered viable pollen grains.

For the in vitro germination test, the experimental design was completely randomized in a 2 x 7 factorial scheme, with 2 storage conditions (environment at 27 ± 2°C and refrigerated at 5 ± 2°C) and 7 different times of conditioning of pollen grains (0, 4, 8, 12, 16, 20 and 24 hours). The experiment consisted of 4 repetitions, each repetition consisting of a slide formed from each Petri dish. The pollen grains of each evaluated slide were observed under the optical microscope, using the 10x and 40x objectives.

For the feasibility tests, the first evaluation was performed (0 hours of packaging) at 8 a.m., shortly after pollen collection. The experimental design was completely randomized in a 2 x 7 factorial scheme, with 2 storage conditions (environment at 27 ± 2°C and refrigerated at 5 ± 2°C) and 7 different times of pollen grain conditioning (0, 4 , 8, 12, 16, 20 and 24 hours). The experiment consisted of 3 repetitions, and each repetition consisted of an inoculated slide. Pollen grains from each slide were observed under the optical microscope using 10x and 40x objectives.

The variables evaluated were subjected to analysis of variance, and the effects of quantitative treatments were submitted to polynomial regression. The analyzes were performed by the computer program R (R Core Team, 2018).

Results and discussion

Determination of floral opening and female, male and senescence stages

Flower bud marking was started at 16:00 hours (h) on the first day of evaluation. The visual observation of the flowers was made every 2h, making a total of 46h of evaluation. During the study there was heterogeneity in the behavior of sugar-apple flowers, with flowers in the female stage and flowers in the male stage in the morning.

The phases of the sugar-apple [closed flower bud, anthesis (female stage), male stage, senescent blossom] observed during the evaluations can be seen in Figure 2.

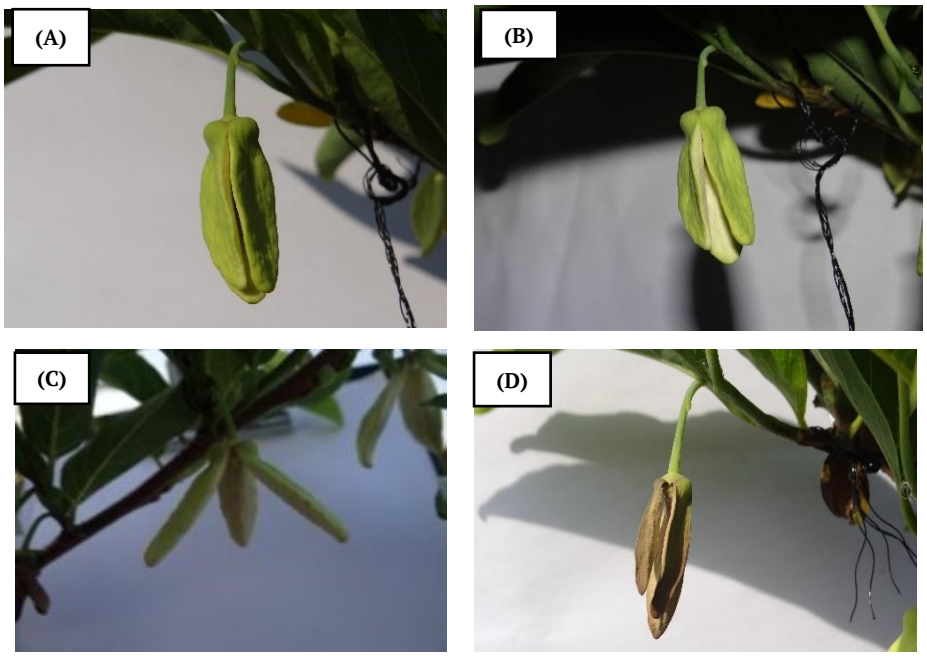

Figure 2. Illustration of the sequence of the flowering stage (Annona squamosa L) from the closed flower bud to senescence in Savannah conditions. Closed flower bud (A), Anthesis (female stage) (B), Fully open flower (Male stage) (C) and Senescence (D).

It was observed that anthesis began at 00:00h (2nd assessment day), in 53% of marked flowers, and extending until 12:00h of the same day, when 100% of flowers reached anthesis (Figure 3). This result is similar to that obtained for flowers of araticum (*Annona crassiflora* Mart.), where the anthesis is gradual and begins in the early hours of the day and may extend until the early hours of the following day (Almeida-Júnior et al., 2018).

The transition from the female stage to the male stage occurred between 6:00 and 12:00 in the morning of the following day (2nd assessment day). In this interval, the vast majority of flowers have reached the male stage. However, a small portion of the flowers of this sample extended until 8:00 a.m. the next day (3rd assessment day) (Figure 3).

This transient phase of female to male flower is characterized by full opening of flowers and anther dehiscence (Ribeiro, São José, Rebouças, & Amaral, 2008). In flowers belonging to the family of Annonaceae, the male stage of protogenic species occurs between 3 and 6 a.m. with the beginning of pollen release (Carvalho & Webber, 2000). Already the female reproductive structure of *A. squamosa* matures first and can remain receptive for up to twenty-four hours (Gazit, Galon, & Podoler, 1982).

It was observed that the number of flowers in the female stage increased from 00:00 in the morning, and the maximum percentage of female flowers was observed at 6 in the morning, probably the most appropriate time to perform the pollination as there is a decrease after this time. The maximum percentage of flowers in the male stage was observed at 10:00 a.m. in the morning.

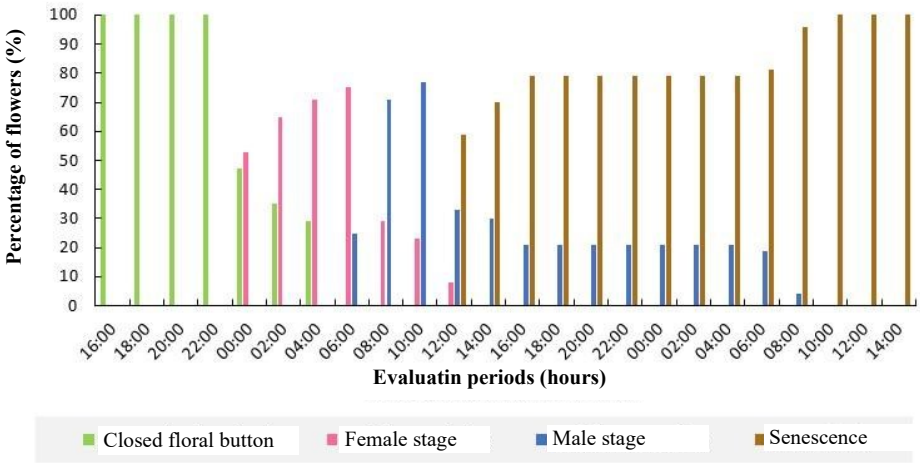

Figure 3. Percentage of sugar-apple (*Annona squamosa* L.) flowers in the stages: Closed flower bud, female stage, male stage and senescence as a function of the observation hours (h).

This information allows to estimate that artificial pollination can be performed in the morning shift, between 02:00 and 06:00 a.m. in tropical and subtropical regions, where climatic factors caused less damage during pollination, and may be extended until 10:00 am, however, with fewer female flowers. However, it is necessary to observe the viability period of pollen grains, because only the period after 06:00 a.m. is when there is viable pollen to perform artificial pollination.

Feasibility of pollen grains

The viability evaluation of pollen grains in minutes showed a decreasing quadratic effect for pollen stored at refrigerated temperature (5 ± 2°C) from 12 o'clock in the afternoon. However, pollen stored under ambient conditions (27 ± 2°C) remained viable until 24h in the afternoon after storage. These results indicate that it is not necessary to store under refrigerated conditions to maintain the viability of the pollen in the studied period (Figure 4).

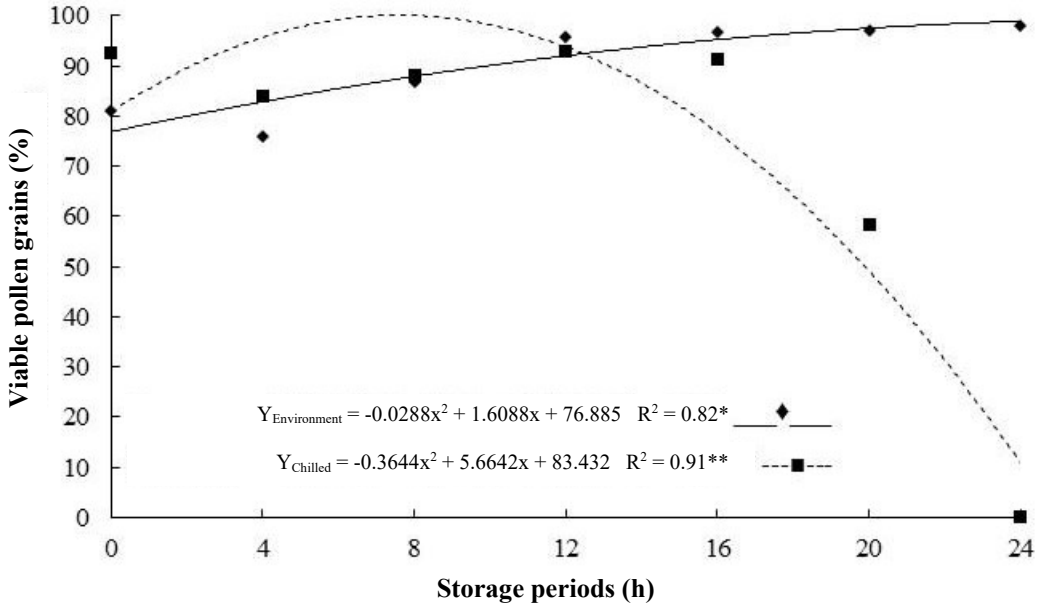

Figure 4. Percentage of viable pollen (*Annona squ amosa* L.) stored under different conditions (ambient pollen: 27 ± 2°C and refrigerated pollen: 5 ± 2°C and storage periods (h). *Significant to ($p < 0.05$); **Significant to ($p < 0.01$).

The unfolding of the second-degree equation indicates that pollen grains may have a 99.35% viability over a period of approximately 28h of storage under ambient conditions (27 ± 2°C). Despite the high initial percentage of viable pollen grains stored in refrigerated environment (5 ± 2°C), reaching 100% viability after 8 hours of storage, this was not considered an adequate environment for storage, indicating that pollen when refrigerated, should be used within 8 hours after storage, losing its viability after this period.

Changes in environmental conditions strongly affect the viability of pollen grains in vitro, and small decreases in relative humidity and temperature increases may indicate significant reductions (Alves Rodrigues, Nietsche, Mercadante-Simões, Toledo Pereira, & Ribeiro, 2018). Pre-anthesis temperatures may also be involved in pollen vigor reduction, impacting on the reduction of accumulated starch in the maturation phase and on the metabolism of the reserves, since most of the time, pollen twinning occurs autotrophically (Baker & Baker, 1979).

In a study on the viability of araticum pollen (*Annona crassiflora*, Mart.) using acetic carmine test under ambient conditions, (Cavalcante, Naves, Franceschinelli, & Silva, 2009) observed a percentage of 93.83%, considered high (Mendes, Costa, Nietsche, Oliveira, & Pereira, 2012), observed that pollen grain viability in seed and seedless accessions was 38.5 and 52.5%, respectively. These results are similar to those found in the present study, where viability was verified above 95% for pollen stored under ambient conditions (27 ± 2°C) using the solution of 2, 3, 5 triphenyl tetrazolium chloride TTC (Figure 5).

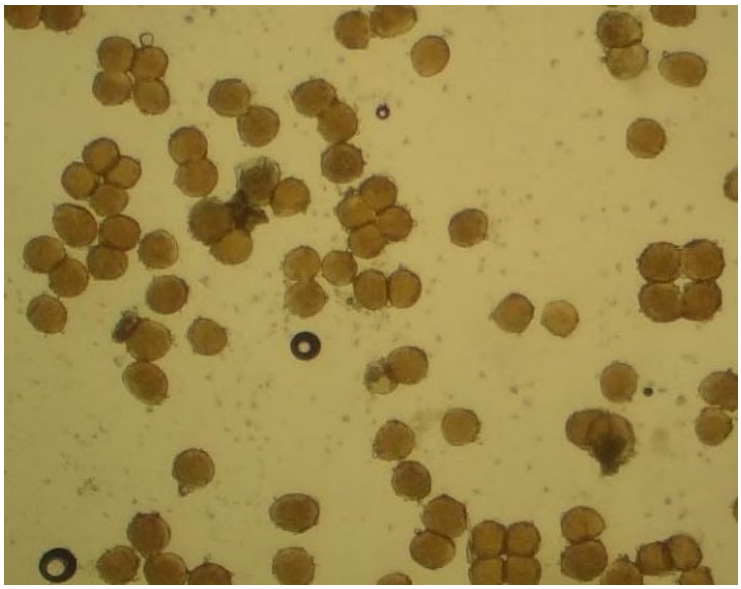

Figure 5. Viability test of *Annona squamosa* L. pollen grains with 2, 3, 5 triphenyl tetrazolium chloride (TTC). Non-viable pollen grains.

According to (Nascimento, Gomes, Batista, Freitas, 2012), pollen grains with low viability generally result in low fruit fixation. Therefore, determining the viability of pollen grains is important as it contributes to the practice of artificial pollination, increasing productivity and quality of production.

Pollen viability can be determined by a number of techniques, the most used being in vitro germination (Soares et al., 2008). Pollen viability estimates are one of the most significant instruments in the qualitative evaluation of the materials to be used in the crossings (Wondracek- Lüdke, Custodio, Simpson, & Valls, 2015). Figure 5 shows viable shortcut pollen grains when subjected to the staining test using 2, 3, 5 triphenyl tetrazolium chloride (TTC).

Germination of pollen grains

Significant differences were observed for germination percentage at the 1% level ($p < 0.01$). The sugar apple pollen grains stored under ambient (27 ± 2°C) and refrigerated (5 ± 2°C) conditions presented excellent germination rates at the first evaluation, i.e. storage time 0, with 65.75 and 50.25% respectively. In the first 4 hours of storage, the germination percentage decreased, however, with still satisfactory values, with 51.25 and 45%, with a significant decrease in the later hours. Pollen tube germination data (Figure 6) show a significant drop when compared to viability data (Figure 5).

Results similar to those reported by (Nietsche, Pereira, Oliveira, Dias, & Reis, 2009) and (Pereira, Crane, Montas, Nietsche, & Vendrame, 2014), who reported that the use of pollen shortly after collection presents satisfactory results, were also obtained in the present work. These results confirm that the use of newly collected pollen presents better viability characteristics, culminating in high fruit fixation rates through artificial pollination.

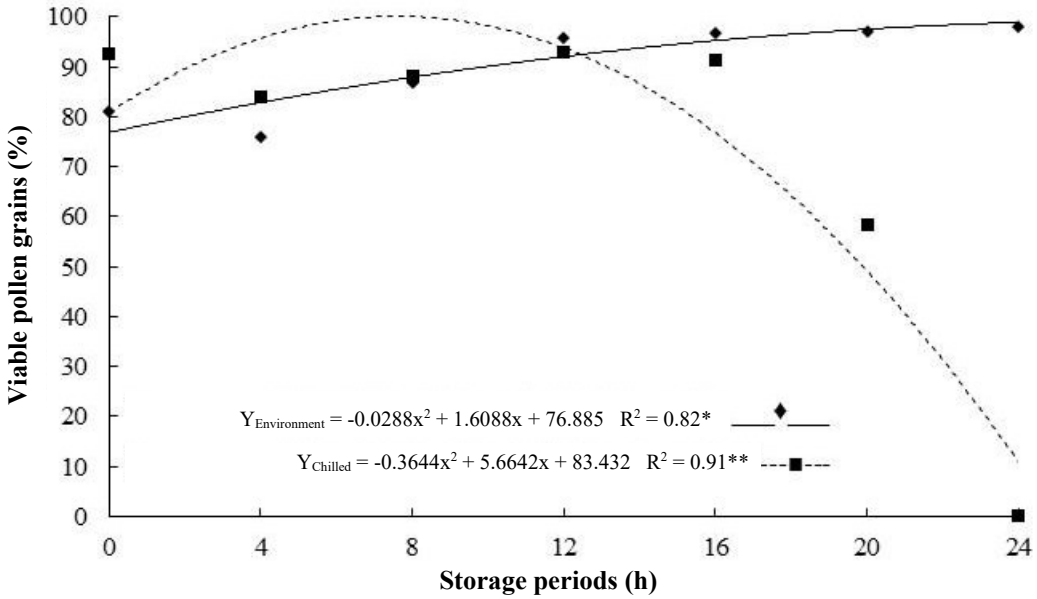

Figure 6. Percentage of germinated sugar apple (*Annona squamosa* L.) pollen grains stored under different conditions (ambient pollen: 27 ± 2°C and refrigerated pollen: 5 ± 2°C and storage periods (h). **Significant to ($p < 0.01$).

On the other hand, Baker and Baker (1979) point out that variations in pre-anthesis temperature alter vigor and affect the accumulated starches during maturation and reserve metabolism. This was confirmed by Lora, Herrero and Hormaza (2012) who reported that starch decomposition and germination of *A. cherimola* pollen grain were affected by pre-anthesis temperatures, with starch loss occurring before anthesis in flowers stored at 25°C, however, there was no effect on storage at 15°C. Similarly Matsuda, Higuchi and Ogata (2016) observed that germination decreased below 14°C, being more noticeable with restrictive effect of pollen tube below 6°C and causing pollen wilting at 4°C, with the same effects occurring above 27°C, with only the pre-anthesis temperature in the range of 20-22°C ensuring increased pollen grain germination.

Mendes et al. (2012) obtained similar results for 'Brazilian seedless' sugar-apple pollen grain germination *in vitro* , reaching 52.5% at a controlled temperature of 25 ± 1°C stored for 6 hours. Rodrigues et al. (2016 obtained in vitro germination of *A. squamosa* from 48.13% at 25 ± 1°C. Evaluating pollen germination in 'Red' and 'Thai Lessard' sugar apple cultivars and in the 'Gefner' atemoya hybrid in tropical conditions, Pereira et al. (2014) obtained low germination percentages, with 26.5, 35.6 and 37.2% respectively. Under semiarid climatic conditions, Alves Rodrigues et al. (2018) obtained a total germination percentage of 60.4% at 26.9°C.

The success of in vitro germination of pollen grains depends on several endogenous and exogenous factors such as plant nutritional status, time and method of pollen grain collection, photoperiod, environmental variations, incubation period and composition of the medium of culture (Alves Rodrigues et al., 2018; Chagas et al., 2010; Ramos, Pasqual, Salles, Chagas and Pio, 2008; Soares et al., 2008; Souza, Souza, Silva, Barbosa, & Araújo, 2014). It is also important to emphasize that the culture medium factor is specific for each species (Dafni, 1993), so this is a component that must be rigorously studied for successful in vitro germination.

The storage of pollen grains in refrigerated environment may or may not promote good conditions for germination. Bettiol Neto, Del Nero, Kavati and Pinto-Maglio (2009) in in vitro germination tests and field pollination of cherimóia, sugar-apple and atemoya, found that pollen samples collected in the humid period and stored in the refrigerator were the ones with the best germination rates under temperate conditions. For the present study with sugar apple under tropical climate conditions, pollen grains under refrigerated storage (5 ± 2°C) presented a percentage below 45% after 4 hours of storage, drastically decreasing after this period, not being considered a satisfactory germination percentage after this period (Figure 6).

Despite the existence of publications in which colorimetric tests are used as a pollen vigor parameter (Cabral, Rossi, Klein, Vieira, & Giustina, 2013; Hister & Tedesco, 2016; Nunes, Bustamante, Techio, & Mittelmann, 2012), colorimetry should not be used as the sole indication of pollen viability because it can only point to the presence of cellular content, which does not necessarily imply the formation of the pollen tube and subsequent fertilization. In vitro tests of pollen tube germination are required to prove this viability more safely. Figure 7 shows the in vitro germination test of sugar apple pollen grains in the present experiment.

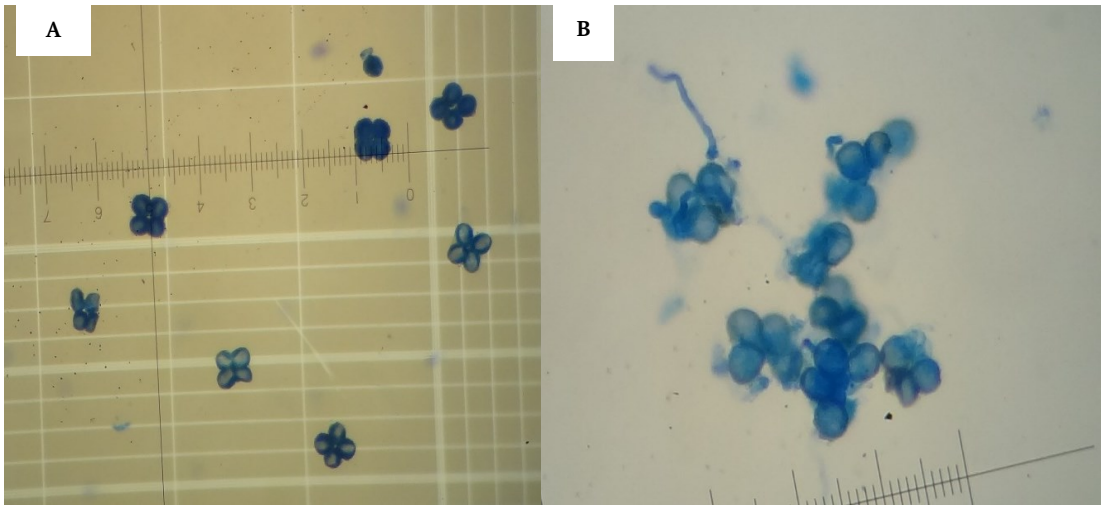

Figure 7. In vitro pollen seed germination test (*Annona squamosa* L.). (A) Tetrad pollen grains - beginning of germination. (B) Germinated pollen grains.

Conclusion

The anthesis of the flowers (female stage) of the sugar-apple begins at 00:00, extending until 12:00 of the same day. The flowers of the sugar apple reach a functionally staminate stage (male stage) from 6 a.m. in the tropical climate conditions.

Pollen grains stored at ambient conditions (27 ± 2°C) remain viable for up to 24 hours after collection, showing that storage in a cold environment at low temperatures (5 ± 2°C) is not necessary.

The sugar apple pollen grains have a germination percentage of 51.25% when stored under ambient conditions (27 ± 2°C) for up to 4 hours after collection.

References

Almeida-Júnior, E. B., Collevatti, R. G., Telles, M. P. C., Chaves, L. J., Neres, D. F., & Soares, T. N. (2018). Short-distance pollen dispersal in a protogynous Annonaceae tree species from the Brazilian Cerrado. *Plant Systematics and Evolution*, *304*(9), 1091-1099. doi: 10.1007/s00606-018-1534-z

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Moraes Gonçalves, J. L., & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift, 22*(6), 711-728. doi: 10.1127/0941-2948/2013/0507

Alves Rodrigues, B. R., Nietsche, S., Mercadante-Simões, M. O., Toledo Pereira, M. C., & Ribeiro, L. M. (2018). Climatic seasonality influences the development of pollen grains and fruiting in *Annona squamosa*. *Environmental and Experimental Botany, 150*, 240-248. doi: 10.1016/j.envexpbot.2018.03.025

Araújo, W. F., Andrade Junior, A. S., Medeiros, R. D., & Sampaio, R.A. (2001). Precipitação pluviométrica mensal provável em Boa Vista, estado de Roraima, Brasil. *Revista Brasileira de Engenharia Agrícpça e Ambiental, 5*(3), 563-567. doi: 10.1590/S1415-43662001000300032

Baker, H. G., & Baker, I. (1979). Starch in angiosperm pollen grains and its evolutionary significance. *American Journal of Botany, 66*(5), 591-600. doi: 10.1002/j.1537-2197.1979.tb06262.x

Bettiol Neto, J. E., Del Nero, M., Kavati, R., & Pinto-Maglio, C. A. F. (2009). Viabilidade e conservação de pólen de três anonas comerciais. *Bragantia, 68*(4), 825-837. doi: 10.1590/S0006-87052009000400002

Brewbaker, J. L., & Kwack, B. H. (1963). The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany, 50*(9), 859-865. doi: 10.1002/j.1537-2197.1963.tb06564.x

Cabral, J. C., Rossi, A. A. B., Klein, M. E., Vieira, S., & Giustina, L. D. (2013). Estimativa da viabilidade polínica em acessos de *Theobroma cacao* L. Baseada em testes calorimétricos. *Enciclopédia Biosfera, 9*(17), 2780-2788.

Carvalho, R., & Webber, A. C. (2000). Floral biology of *Unonopsis guatterioides* (A. DC.) R.E. Fr. an Annonaceae pollinated by Euglossini. *Brazilian Journal of Botany, 23*(4), 421-425. doi: 10.1590/S0100-84042000000400008

Cavalcante, T. R. M., Naves, R. V., Franceschinelli, E. V., & Silva, R. P. (2009). Pollination and fruit development in araticum. *Bragantia, 68*(1), 13-21. doi: 10.1590/S0006-87052009000100002

Chagas, E. A., Pio, R., Chagas, P. C., Pasqual, M., & Bettiol Neto, J. E. (2010). Composição do meio de cultura e condições ambientais para germinação de grãos de pólen de porta-enxertos de pereira. *Ciência Rural, 40*(2), 231-236. doi: 10.1590/S0103-84782010000200002

Dafni, A. (1993). *Pollination ecology: a practical approach*. Oxford UK; New York, NY: Oxford University Press.

Einhardt, P. M., Correa, E. R., & Raseira, M. C. B. (2006). Comparação entre métodos para testar a viabilidade de pólen de pessegueiro. *Revista Brasileira de Fruticultura, 28*(1), 5-7. doi: 10.1590/S0100-29452006000100004

Ferreira, C. A., Pinho, É. V. D. R. V., Alvim, P. D. O., Andrade, V. D., Silva, T. T. D. A., & Cardoso, D. L. (2007). Conservação e determinação da viabilidade de grão de pólen de milho. *Revista Brasileira de Milho e Sorgo, 6*(2). doi: 10.18512/1980-6477/rbms.v6n02p%25p

Gazit, S., Galon, I., & Podoler, H. (1982). The role of nitidulid beetles in natural pollination of annona in Israel. *Journal of the American Society for Horticultural Science, 107*(5), 849-852.

George, A. P., & Nissen, R. J. (1988). The effects of temperature, vapour pressure deficit and soil moisture stress on growth, flowering and fruit set of custard apple (*Annona cherimola* × *Annona squamosa*) 'African Pride'. *Scientia Horticulturae*, *34*(3-4), 183-191. doi: 10.1016/0304-4238(88)90091-X

Hister, C. A. L., & Tedesco, S. B. (2016). Estimativa da viabilidade polínica de araçazeiro (*Psidium cattleianum* Sabine) através de distintos métodos de coloração. *Revista Brasileira de Plantas Medicinais, 18*(1), 135-141. doi: 10.1590/1983-084X/15_081

Khalate, S. M., Supe, V. S., & Doke, N. D. (2018). Effect of cover crops on pollen viability and fruit set in custard apple (Annona squamosa L.). *Trends in Biosciences*, *11*(6), 839–841.

Wondracek-Lüdke, D. C., Custodio, A. R., Simpson, C. E., & Valls, J. F. M. (2015). Crossability of Arachis valida and B genome Arachis species. *Genetics and Molecular Research*, *14*(4), 17574–17586. doi: 10.4238/2015.December.21.30

Linsley, E. G. & Cazier, M. A. (1963.) Further observations on bees which take pollen from plants of the genus *Solanum*. *Pan-Pacific Entomology*, *39*, 1-18.

Liu, K., Yuan, C., & Jing, G. (2013). Effect of exogenous oxalic acid treatment on ripening and preservation of *Annona squamosa* L. fruits during postharvest storage. *Food Science, 14*, 329-334.

Liu, K., Li, H., Yuan, C., Huang, Y., Chen, Y., & Liu, J. (2015). Identification of phenological growth stages of sugar apple (*Annona squamosa* L.) using the extended BBCH-scale. *Scientia Horticulturae, 181*, 76-80. doi: 10.1016/j.scienta.2014.10.046

Loguercio, L. L. (2002). Pollen treatment in high osmotic potential: a simple tool for in vitro preservation and manipulation of viability in gametophytic populations. *Brazilian Journal of Plant Physiology, 14*(1), 65-70. doi: 10.1590/S1677-04202002000100009

Lora, J., Herrero, M., & Hormaza, J. I. (2012). Pollen performance, cell number, and physiological state in the early-divergent angiosperm Annona cherimola Mill. (Annonaceae) are related to environmental conditions during the final stages of pollen development. *Sexual Plant Reproduction, 25*, 157-167. doi: 10.1007/s00497-012-0187-2

Lorenzon, M. C. A., & Almeida, É. C. (1997). Viabilidade e germinação do pólen de linhagens parentais de cebola híbrida. *Pesquisa Agropecuária Brasileira, 32*(4), 345-349. Recovered from https://seer.sct.embrapa.br/index.php/pab/article/view/4661/0

Madhu, C., Brainard, P. J., Raj, G. P., Swapn, J., & Rao, A. S. S. (2012). Anti ulcer activity of aqueous extract of *Annona squamosa* leaves on rats. *International Journal of Pharmaceutical Sciences and Research, 3*(11), 4429-4433. Recovered from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.278.4047&rep=rep1&type=pdf

Manvi, F. V., Nanjwade, B., & Shing, S. (2011). Pharmacological screening of combined extract of *Annona squamosa* and *Nigella sativa*. *International Journal of Pharma and Bio Sciences, 2*(2), 520-529.

Matsuda, H., Higuchi, H., & Ogata, T. (2016). Anatomical observations of pollen starch accumulation and pollen germinability as affected by pre-anthesis night temperatures in cherimoya (*Annona cherimola* Mill.). *Tropical Agriculture and Development, 60*(3), 155-161. doi: 10.11248/jsta.60.155

Mendes, H. T. A., Costa, M. R., Nietsche, S., Oliveira, J. A. A., & Pereira, M. C. T. (2012). Pollen grain germination and fruit set in 'Brazilian seedless' sugar apple (*Annona squamosa* L.). *Crop Breeding and Applied Biotechnology, 12*(4), 277-280.

Nascimento, W. M., Torres, A. C., & Lima, L. B. (2003). Pollen viability in hybrid seed production of eggplant under tropical conditions. *ISHS Acta Horticulturae, 607*, 37-39. doi: 10.17660/ActaHortic.2003.607.4

Nascimento, W. M., Gomes, E. M. L., Batista, E. A., Freitas, R. A. (2012). Utilização de agentes polinizadores na produção de sementes de cenoura e pimenta doce em cultivo protegido. *Horticultura Brasileira, 30*(3), 494-498. doi: 10.1590/S0102-05362012000300023

Nietsche, S., Pereira, M. C. T., Oliveira, C., Dias, M. M., & Reis, S. T. (2009). Viability of the sugar apple (Annona squamosa) pollen grains at different hours of the day. *Ciência e Agrotecnologia, 33*(2), 527-531. doi: 10.1590/S1413-70542009000200026

Nunes, R. C., Bustamante, F. O., Techio, V. H., & Mittelmann, A. (2012). Morphology and pollen viability of *Lolium multiflorum* Lam. *Ciência e Agrotecnologia, 36*(2), 180-188. doi: 10.1590/S1413-70542012000200006

Pereira, M. C. T., Crane, J. H., Montas, W., Nietsche, S., & Vendrame, W. A. (2014). 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*(23), 55-60. doi: 10.1016/j.scienta.2014.08.004

Ramos, J. D., Pasqual, M., Salles, L. A., Chagas, E. A., & Pio, R. (2008). Receptividade do estigma e ajuste de protocolo para germinação in vitro de grãos de pólen de citros. *Interciencia Caracas, 33*(1), 51-55. Recovered from https://www.redalyc.org/pdf/339/33933109.pdf

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, AT: R Foundation for Statistical Computing.

Ribeiro, G. S., São José, A. R., Rebouças, T. N. H., & Amaral, C. L. F. (2007). Aspectos da biologia floral relacionados à produção de frutos de pinha (*Annona squamosa* L.). *Acta Scientiarum. Biological Sciences, 29*(4), 369-373. doi: 10.4025/actascibiolsci.v29i4.875

Rodrigues, B. R. A., Santos, R. C., Nietsche, S., Mercadante-Simões, M. O., Cunha, I. R. G., & Pereira, M. C. T. (2016). Determination of cardinal temperatures for sugar apple (*Annona squamosa* L.). *Ciência e Agrotecnologia, 40*(2), 145-154. doi: 10.1590/1413-70542016402039115

Seffrin, R. C., Shikano, I., Akhtar, Y., & Isman, M. B. (2010). Effects of crude seed extracts of *Annona atemoya* and *Annona squamosa* L. against the cabbage looper, *Trichoplusia ni* in the laboratory and greenhouse. *Crop Protection, 29*, 20-24. doi: 10.1016/j.cropro.2009.09.003

Sinimbú Neto, F. D. A., Martins, A. B. G., & Barbosa, J. C. (2011). Viabilidade 'in vitro' de grãos de pólen de bacurizeiro - Clusiaceae. *Revista Brasileira de Fruticultura, 33*(2), 593-600. doi: 10.1590/S0100-29452011005000062

Soares, T. L., Silva, S., Costa, M., Santos Serejo, J., Souza, A., Morais-Lino, L., ... Jesus, O. (2008). In vitro germination and viability of pollen grains of banana diploids. *Crop Breeding Applied Biotechnology, 8*, 111-118.

Souza, G. S., Souza, Z. M., Silva, R. B., Barbosa, R. S., & Araújo, F. S. (2014). Effects of traffic control on the soil physical quality and the cultivation of sugarcane. *Revista Brasileira de Ciência do Solo, 38*(1), 135-146. doi: 10.1590/S0100-06832014000100013

Zucareli, V., Ferreira, G., Silvério, E. R. V., & Amaro, A. C. E. (2007). Luz e temperatura na germinação de sementes de *Annona squamosa* L. *Revista Brasileira de Biociências, 5*(2), 840-842.