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Floral biology and Implications for Apple Pollination in Semiarid Northeastern Brazil

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Abstract

The ongoing interest in apple production has increased the search for new varieties and growing areas. Varieties with lower requirements concerning chilling and subjection to chemical and/or physical treatments to break down floral dormancy has enabled the expansion of this cultivation in tropical areas. We evaluated the impact of temperature on floral biology of the varieties Julieta (pollen donor) and Princesa (pollen recipient) grown in semiarid Northeastern Brazil. Flowers of the Julieta had lower lifespan than those of the Princesa (*Julieta*= 2.04 ± 0.197; *Princesa*2.93 ± 0.274 days; Mann-Whitney*U*= 150, p<0.0001). Pollen release in the Julieta occurred from 09:00h on the first day, with peak at 13:00h on the second day. Stigma receptivity in the Princesa began during the "balloon" stage and remained until senescence. There was an average loss of 19.87%± 15.79 in the number of anthers of the Julieta that effectively released pollen compared to the total amount produced by flower, representing a decrease in pollen availability for pollination. The average temperature of 30.21ºC ± 4.18 may have been responsible for the decrease in longevity and the loss of anthers and pollen, thus it is recommended to increase the number of pollen donor plants in semiarid environments.

Keyword: Climate constrain. Floral phenology. Malusdomestica. Pollination biology. Tropical agriculture

Introduction

The increasing demand for food combined with the predictions on climate change has led many producers to expand geographically the cultivated areas, including other regions with environmental conditions hitherto considered unsuitable (Miranda et al., 2013). Due to the implementation of new technologies, environmental impacts have been minimized, making possible the establishment and cultivation of exotic plants (Fachinello et al., 2011). Production of deciduous fruit trees, for example, is increasing in the tropics in recent decades (Puentes et al., 2008; Patiño; Miranda, 2013). In tropical regions, production of these fruits can be influenced by climatic conditions that limit not only the potential for growth, but even can make cultivation impossible for certain species (Fischer, 2012). For species of the family Rosaceae, higher temperatures and/or other environmental stress negatively affect pollen and pistil, and may promote a reduction in adherence rates, germination of pollen grains and stigma receptivity (Young et al., 2004, Koti et al., 2005, Hedhlyet al., 2003, 2005).Floral induction may also be changed by increasing temperature and solar radiation, as reported by Kurokura et al. (2013).

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Apple trees belong to the family Rosaceae, subfamily Maloidea and genus *Malus*, being a fruit treewith the highest importance worldwide, considered one of the main agricultural products (FAO, 2014*). Malusdomestica* Borkhis a hybrid coming from several species of this genus (Hokanson et al., 2001) that presents high self-incompatibility rates (Soster; Latorre, 2007). Its cultivation requires the planting of more than one variety that present synchronous flowering (Kevan, 2010). In a commercial orchard, varieties of pollen donors and recipients areused for cross-pollination between varieties, increasing the rates of ripening and production (Kevan, 2010). Apple trees are originally from temperate climate, and for the occurrence of budding and flowering the vegetative gems need to be subjected to low temperatures, below 7.2° C. However, higher temperatures may also be effective for the opening of the buds (Fischer, 2012). In recent decades, breeding programs have developed cultivars with low chilling requirements and in concomitant action on the chemical and physical processes such as the application of hormones, the defoliation of the plant and the water stress (Ramírez; Kallarackal, 2014),have enabled the cultivation in other regions with higher temperatures. Nevertheless, where chilling is not ideal, physiological abnormalities may be observed, as low sprouting and formation of lateral buds, extended flowering period, low formation of flower buds and low fruit productivity (Fischer, 2012). In Brazil, where the production area has expanded to warmer regions (Patiño; Miranda, 2013), usually the number of chill hours required by the varieties does not reach the minimum required for spontaneous flowering, demanding other chemical and/or physical treatments to break dormancy (Webster, 2005). The knowledge of the biological behavior of plants in environments different from those where they are usually grownis indispensable to the development of agricultural practices that allow the potentiation in its production (Valentiniet al., 2001;Miranda et al., 2013). Herein, we studied the floral development, production and availability of tree pollen in order to evaluate possible limiting factors to the pollination of this crop in semi-arid Northeastern Brazil.

Material and Methods

1.1 Study Area

We developed the study during the period of induced flowering, between October and December 2013, in the experimental cultivation of the FrutaCorfarmlocated in Tabuleiros de Russas Irrigate Perimeter (04°57'46.1'' S and 38°02'35'' W), in the lowerportion of the Jaguaribe river, 170km far from Fortaleza, Ceará State, Brazil. We evaluated specimens of *M. domestica*with 30 months of age, distributed on an area of 0.5 hectares. The original area was represented predominantly by soil type red-yellow Podzolic, with vegetation cover formed by dense shrubby caatinga. The climate of the region is warm tropical semi-arid (Köppen, 1949), with average temperature between 26 and 28ºC and accumulated rainfall of 857.7 mm, concentrated between February and April.

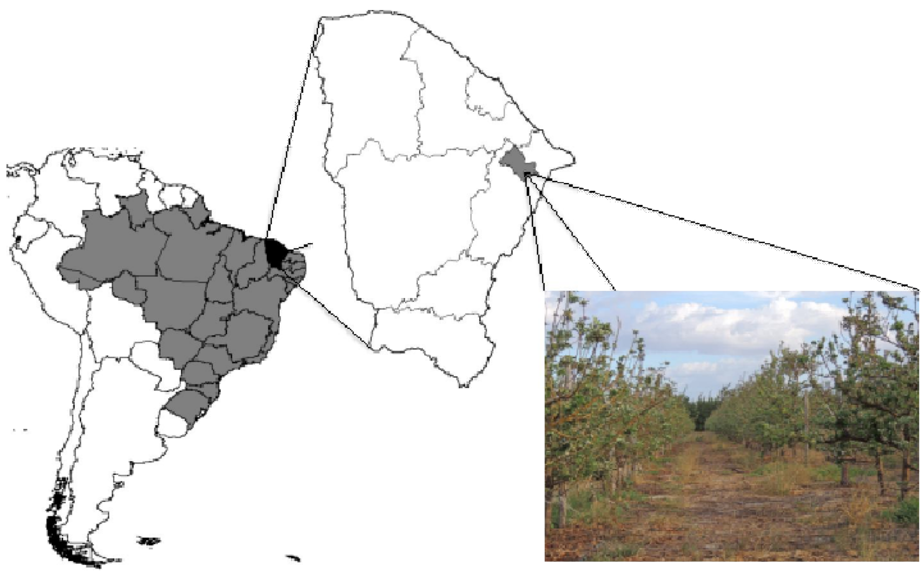

Figure1: Study area. Farm in the city of Russas, in the Mesoregion of the Jaguaribe, Ceará State. B: Orchard Overview

1.2 Varieties Studied

We studied two of the six varieties of *M. domestic* grown in the farm; var. Princesa, developed by the Experimental Research Station of Santa Catarina (EPAGRI) and var. Julieta, developed by the Agronomic Institute of Paraná (IAPAR), the first being pollen recipient, and the second, pollen donor. These varieties have been selected due to the positive response to the chemical and physical treatment for floral induction, fruiting and rusticity under the studied conditions.

1.3 Aspects of the Floral Biology

We evaluated, for each of the varieties, 50 individuals and in each of them, at random, we marked an inflorescence. In each of these inflorescences, we counted the number of flowers and randomly marked a floral bud at the "balloon" stage and monitored it until flower senescence. We evaluated the following floral events: the time of an thesis and petals movements, the opening of the anther and pollen release, stigma receptivity and floral longevity. To evaluate the an thesis, we followed the 50 flower buds until the total expansion of the petals. We observed such buds throughout the day, every two hours, from05:00 amto 05:00 pm and continued in subsequent days until the time of an thesis. In the same floral buds of both varieties, we observed the time of opening of anthers and pollen release with the aid of hand magnifying glass. To evaluate the stigma receptivity, we marked 35 floral buds at the "balloon" stage only in the recipient variety, Princesa. To avoid the deposition of self-pollen grains on the stigma, which would cause a reaction with hydrogen peroxide, we carefully opened the buds and made the emasculation of anthers with the aid of scissors. We kept the buds isolated with tulle bags, and we evaluated the stigma receptivity through the application of hydrogen peroxide solution (3%) (Dafni, 1992) always at the same time of the day, c.a. 10:00a.m.To this end, the stigma was immersed in an Eppendorf tube containing the solution, and if receptive, bubbles of oxygen were formed. The 35 buds were divided into seven groups; one group assessed per day, totaling five evaluation days. We applied the Mann-Whitney test (U) to determine if there were differences between (i) the number of flowers per inflorescence, (ii) the number of anthers per flower and (iii) the flower longevity between the varieties studied. The same test was applied to identify the number of anthers at the final stage of the flower`s life is lower than the number of anthers at the initial stage. All statistical analyses were performed using the software Past 3.x.

Results and Discussion

In the semi-arid region of the Ceará State, both *M. domestic* varieties studied started to flower seven days after the treatment to break dormancy, manual defoliation and application of plant hormone. Compared to the studies of Roberto et al. (2006) and Chagas et al.(2012) developed in Southern Brazil, our results showed that the response to treatments to break bud dormancy in the semi-arid region was significantly faster. These authors found flowers from 50 and 30 days after treatment, respectively. Possibly the high temperature and lower altitude may have changed the organic functions in the varieties of *M. domestica* studied, leading to a faster response to bud dormancy break. The flowers of the two studied varieties are usually actinomorphic, hermaphrodite, pentamers with five stilettos united in the base, one inferior ovary containing five carpels with two ovules each. Color varies from dark pink, in buds at "balloon" stage, to light pink for third-day flowersin the var. Princesa (Figure2A-F),and white-pink in buds at “balloon” stage, to white soon after an thesis in the var. Julieta (2G-N).Despite the climatic conditions in the semi-arid, flowers of both varieties were within the standards found in temperate regions(Pratt,1988;Janicket al., 1996;Pereira-Lorenzo et al., 2009).

Figure 2: Flower cycle in the studied varieties of*M.domestica*; Princesa (A-F) and Julieta (G-L). A and G: "balloon" floral buds. B and H: onset of an thesis. C and I: flower with closed anthers. D and J: flowers with open anthers. E and K: flowers at the onset of senescence. F and L: senescence

There were no differences between the varieties studied in production of flowers per inflorescence (Mann-Whitney U = 1145, p = 0.3690) (Figure3A). Our results corroborate those of Racskó; Miller (2010), which suggest that the commercial varieties usually present an average of five flower per inflorescence. Freitas (1995) analyzed seven varieties of *M. domestica*in temperate climate and observed the development of four to seven flowers per inflorescence. This suggests that cultivation at higher temperatures, as the semi-arid, does not necessarily affect the production of flowers. On the other hand, Petri et al. (2006), in Southern Brazil, found a lower number of flowers, an average of three flowers per inflorescence, in apple trees that did not reach their chilling requirements. Although the lack of differences in the production of flowers per inflorescence between the varieties, and comparing with those grown in temperate climates, we detected a significant difference in the number of anthers produced by flower (Mann-Whitney U= 608.5, $p < 0.001$) between the varieties; the var. Princesa (pollen recipient) presented a higher number of anthers per flower (Dall'orto et al.1985, Janick et al.1996, Luchi 2002; Albuquerque Junior et al.2010).

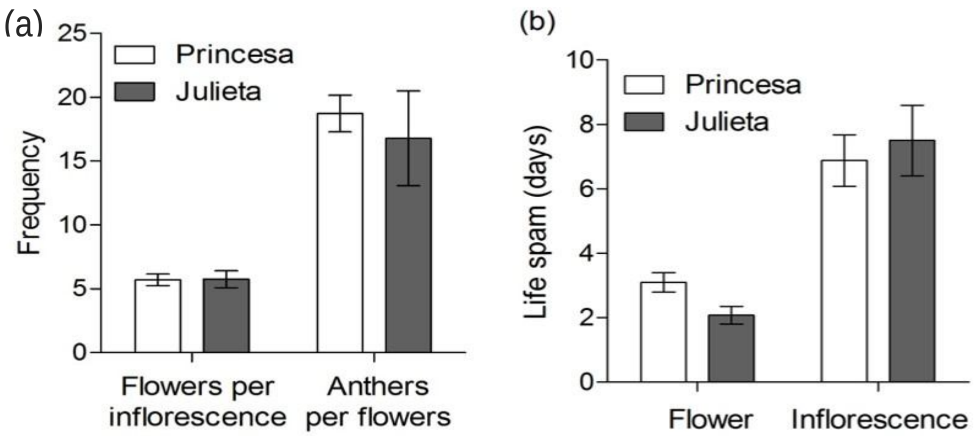

Figure 3. Floral characteristics of the var. Princesa and Julieta of *M. domestic* grown in the semi-arid Northeastern Brazil, Russas, Ceará State. A: average number of flowers per inflorescence and anthers in flowers for each variety studied. B: average lifespan (days) of flower and inflorescence for each variety studied

Considering pollen production, the average number of anthers releasing pollen per flower in the varieties Princesa and Juliet were 18.76 ± 1.378 and 16.8 ± 3.681, respectively. More than 80% of the anthers released pollen up to 28 hours after the an thesis (Figure 4). In this study, we verified that on the donor var. Julieta, pollen release occurred from 09 a.m. of the first day, with a peak at 01 p.m. of the next day (Figure 4), representing other difference when compared to temperate regions, where anthers start dehiscence late in the afternoon (Free, 1993; Freitas 1995).

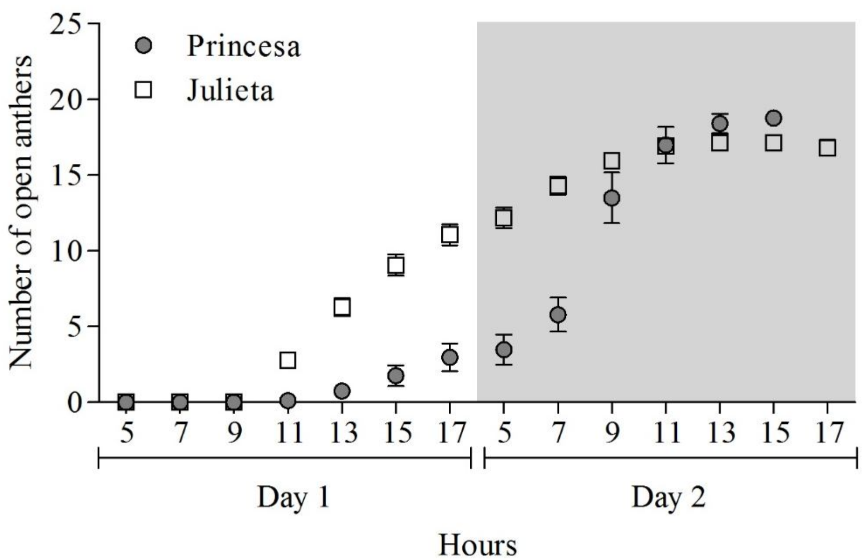

Figure 4: Pollen Release in Flowers of the Varietiesjulieta and Princesa (*M. domestica*) grown in the Semi-arid Northeastern Brazil, Russas, Ceará State

We also observed a decrease in the number of anthers during the flower lifespan, considering from the onset of an thesis until senescence. The var. Julieta, pollen donor, lost, in percentage, almost the double of the anthers when compared to the var. Princesa ($var_{Princesa}$ = 19.87 % ± 10.82; $var_{Julieta}$ = 9.61% ± 15.79; Mann-Whitney U= 736.5; p = 0.0003) (Figure 5).These losses are related to the early detachment of the anther from the flower, before pollen release, and to the presence of burning and nonviable pollen from an thesis to senescence.

Figure 5: Damage Caused by High Temperature Few Hours after Anthesis of Flowers Of*m. Domestica* grown in the Semi-Arid Northeastern Brazil, Russas, and Ceará State

Concerning stigma receptivity, the stigma ofthe var. Princesa was already receptive in flower buds at "balloon" stage, corroborating the results of Losada; Herrero (2012). In relation to the duration of receptivity, stigmas were receptive for up to 3 days after an thesis, one day lesser than the results found by Freitas (1995) in temperate climate. As for the flower lifespan, flowers in the var. Juliet lasted for fewer days than those of the var. Princesa ($Var_{Julieta}$= 2.04 ± 0.197; $Var_{Princesa}$2.93 ± 0.274; Mann-Whitney U = 150, $p < 0.0001$) (Figure 3B). However, in the var. Julieta, the longevity of the inflorescence was, on average, a day longer (Figure3B). The longest duration of the pollen recipient flowers compared to the pollen donor flower increases the chances of being pollinated and consequently the probability of forming fruit (Harder; Johnson, 2005). The total period of flowering in the two varieties studied here, which is 37 days, was longer than that observed in other regions with milder temperatures. In Southe as thern Brazil, Chagas et al. (2012) studied different varieties of *M. domestica*, including the var. Princesa, and reported differences in the flowering period, which varied between 21 to 33 days. The var. Princesa was one of the varieties with longer flowering period, between 30 and 33 days, however, still shorter than the period observed in this study. Our results show that although the studied varieties have been able to flower under adverse environmental conditions in the northeastern semi-arid region, the floral phenology was different from that observed for *M. domestica*in colder climates, including the same variety Princesa (Chagas et al., 2012; Albuquerque Junior et al., 2010). These differences may have significant effects on pollination and crop production, once the apple tree is highly dependent on cross-pollination between the cultivated varieties (Kevan, 2010).When considering this behavior along with shorter lifespan, increased loss of anthers and lower availability of pollen presented by the flowers, the result may be a deficitin pollination.

The most plausible explanation for the observed phenological changes is the high average temperature of the planting site. We observed an average of 30.21 ºC ± 4.18 during the flowering period, temperature even higher than the average for the Northeastern semi-arid region, which varies between 23 and 27°C (Moura et al. 2007). High temperatures can influence the acceleration of metabolic activities in the flowers, leading to an acceleration of life cycle compared to flowers of milder climate regions (Chmielewski et al. 2004).In addition, high temperatures can alter the formation of tissues of the anthers (Zinn et al. 2010) and affect the transport of photo assimilates to the reproductive structures, which may result in high rates of abscission of flower buds and impaired fruiting(Zhao et al. 2005; Snider; Oosterhuis 2012). In the family Rosaceae, which includes theapple tree, researches have shown that higher temperatures and/or other environmental stress are the main factors that negatively affect pollen and pistil (Young et al., 2004; Koti et al., 2005 e Hedhly et al., 2005). Moreover, Petri (2004) argues that environmental conditions have a strong influence on the formation of the anthers of apple trees, and may affect the amount formed from one year to the next. We observed that the anthers in the varieties Princesa and Julieta, with longer exposure to solar incidence, started abscission more rapidly and, depending on the exposure time, the damages were even more severe in the flowers. These physiological processes and their implications for the pollination and fertilization need to be better understood, as Hasanuzzaman et al. (2013) stated that with climate change, plants will be subjected to higher temperatures than those supported presently in nature, which would promote atypical behavior such as acceleration of metabolism as a result of the new conditions imposed by the environment.

In addition, studies have shown that changes in floral morphology, among them a few floral signals, e.g. the color, the volatile compounds and nectar guides, determine the pattern of pollinator visitation and may interfere with pollination (Voguel, 1969; Dafni, 1992; Tuell; Isaacs, 2010). On the other hand, increasing temperature and solar radiation imply significantly increased emission of volatiles associated with the attraction of pollinating insects (Jakobsen; Olsen, 1994).Even after pollination, temperatures above 30ºCmay dramatically affect the reproduction of several cultivated species, especially fertilization, through the inhibition of the development of male and female gametophytes, disruption of germination of pollen grains, limiting pollen tube growth, among others (Kakani et al., 2005; Hedhly et al., 2005; Jain et al., 2007; Hedhly et al., 2009; Snider et al., 2009, 2011a,b,c,d; Snider; Oosterhuis, 2012; Snider; Kawakami, 2014). Finally, our study demonstrated that the two varieties have reached reproductive stage and produced viable flowers. However, the environmental conditions of the semi-arid Northeast, mainly the high temperatures, influenced the floral phenology of varieties of the studied apple tree by reducing the longevity of flowers and the availability of pollen. According to Blümel (2015), the reproductive success of plants is related to the complex network of epigenetic factors and their interaction with external stimuli. Furthermore, the cultivation of species originally from temperate climate in tropical environments has become a reality, and the climate change ahead will make cultivation at higher temperatures an increasing need (Hasanuzzaman et al. 2013). Therefore, further studies are required to develop varieties better adapted to tropical conditions, as well as new planting arrangements, which may compensate for the lower availability of pollen observed at the time.

Conclusions

The results obtained allow us to conclude that the varieties of apple trees grown in the semi-arid region of the Ceará State have favorable aspects for their cultivation, with faster response to flowering, requiring seven days after flower induction and higher average floral longevity than expected for *M. domestica*in temperate regions or regions with milder climate. Therefore, high temperatures are favorable in these aspects. On the other hand, we conclude that the high temperature may have been responsible for burning flower and open flowers, besides the loss of anthers and pollen. However, this is a pioneer study evaluating the cultivation of apple tree in the semi-arid Northeastern Brazil, and more studies are needed to assess the long-term yield in response to climatic conditions of the semi-arid region. Finally, the two varieties have a high reproductive potential in the semi-arid, being important to modify certain aspects at planting, e.g. increasing the number of individuals of the var. Julieta, the pollen donor, to compensate for the losses of anthers and pollen, if the producer desires to cultivate this variety as the pollinizer.

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