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Pollination of flowers of the San Andreas strawberry cultivar

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Abstract – The objective of this work was to determine the optimal day for pollinating the flowers of the San Andreas strawberry cultivar after flower anthesis. The study was carried out in two periods (summer and fall), on a commercial farm. A total of 12 flower buds were monitored and photographed in each of the following 11 treatments: manual cross-pollination (MCP) on the first to fifth day after flower anthesis (MCP1 to MCP5), natural pollination (NP) on the first to fifth day after flower anthesis (NP1 to NP5), and self-pollination. After pollination, the flowers were bagged until the development and maturation of the flower receptacle. Subsequently, the fruits were harvested, quantified, and subjected to evaluations to determine biometry, degree of deformity, and the percentage of fertilized achenes. The flowers of the San Andreas cultivar remain open during five days, when the beginning of the development of the flower receptacle can already be observed. The optimal days for strawberry flower pollination through MCP or NP are the first two days after anthesis.

Index terms: *Fragaria* x *ananassa*, flower anthesis, pollination.

Polinização de flores da cultivar de morango San Andreas

Resumo – O objetivo deste trabalho foi determinar o melhor dia para polinização das flores da cultivar de morangueiro San Andreas após a antese floral. O estudo foi realizado em dois períodos (verão e outono), em uma propriedade de plantio comercial. Um total de 12 botões florais foram acompanhados e fotografados em cada um dos seguintes 11 tratamentos: polinização cruzada manual (PCM) de 1 a 5 dias após a antese floral (PCM1 a PCM5), polinização natural (PN) de 1 a 5 dias após a antese floral (PN1 a PN5) e autopolinização (AP). Após a polinização, as flores foram ensacadas até o desenvolvimento e a maturação do receptáculo floral. Em seguida, os frutos foram colhidos, quantificados e submetidos a avaliações para determinação de biometria, grau de deformidade e percentagem de aquênios fertilizados. As flores da cultivar San Andreas permanecem abertas por cinco dias, quando já se pode observar o início do desenvolvimento do receptáculo floral. Os melhores dias para a polinização da flor do morangueiro pela PCM ou pela PN são os dois primeiros dias após à antese.

Termos para indexação: *Fragaria* x *ananassa*, antese floral, polinização.

Introduction

In recent decades, there has been a growing interest in studying crops and agroecosystems to evaluate the economic significance of pollinators (Wolowski et al., 2019). The species *Fragaria* x *ananassa*, commonly known as strawberry, is classified as a crop with a moderate pollinator dependence (Giannini et al., 2015). Although its flowers are hermaphrodite and self-fertilize, a substantial number of its ovules still require fertilization and the presence of herkogamy, meaning that a pollinator is essential to ensure an even pollen distribution across the stigmas (Malagodi-Braga, 2002).

According to Malagodi-Braga (2018), for the production of perfect and undeformed fruits, pollen grains must reach nearly all stigmas, ensuring a complete ovule fertilization. Witter et al. (2014) also concluded that strawberry flowers need to be fully fertilized in order to yield well-formed fruits, observing that, when the ovule is only partially fertilized, the produced strawberries show varying deformation patterns.

Therefore, it is important to investigate the phenological stages of strawberries regarding pollination. Antunes et al. (2006) found that complete flower opening (stage 3) persisted for 4.5 days in the Oso Grande, Tudla, Chandler, and Dover cultivars, coinciding with the pollination stage when the stigmas become receptive to pollen grains from other flowers. When subjecting the Aromas cultivar to the stigma receptivity test, Malagodi-Braga (2002) and Barbosa & Orth (2020) observed that all stigmas showed receptivity on the fourth day. For the San Andreas cultivar, Silva et al. (2020) reported well-formed fruits when monitoring pollination by bees on the initial day of anthesis. However, information on the receptivity of this cultivar and its inflorescence status on the days available for flower visitation is still scarce. Additionally, there is only preliminary (unpublished) data for the receptivity test, suggesting receptivity starting on the first day of anthesis.

Therefore, considering that the San Andreas cultivar is one of the most cultivated and commercialized in the market (Klein et al., 2020), there is a compelling need to understand its floral phenology and pollination dynamics. Determining these details is crucial in genetic improvement research and can significantly contribute to future investigations.

The objective of this work was to determine the optimal day for pollinating the flowers of the San Andreas strawberry cultivar after flower anthesis.

Materials and Methods

The study was conducted in a commercial strawberry farm, in the municipality of Barra do Choça, located in the Planalto da Conquista region, in the state of Bahia, Brazil (14º52'S, 40º34'W, at an average altitude of 900 m). The area has an average annual rainfall of approximately 750 mm, with a dry season from May to September according to the data obtained, in 2020, at the meteorological station of Universidade Estadual do Sudoeste da Bahia.

For the study, the San Andreas cultivar was cultivated in an open field under a low-tunnel protection, using fertigation as needed. The experiment covered two periods, the summer (January) and fall (April) of 2022, under continuous flowering (Figure 1). The evolution of the floral characteristics of the cultivar was monitored throughout a five-day experiment. For this, 12 flower buds were meticulously observed and photographed in each of the following 11 treatments: manual-cross pollination (MCP) on the first to fifth day of flower anthesis (MCP1 to MCP5), natural pollination (NP) on the first to fifth day of flower anthesis (NP1 to NP5), and self-pollination (SP). The treatments were conducted in beds, comprising two rows and a total of

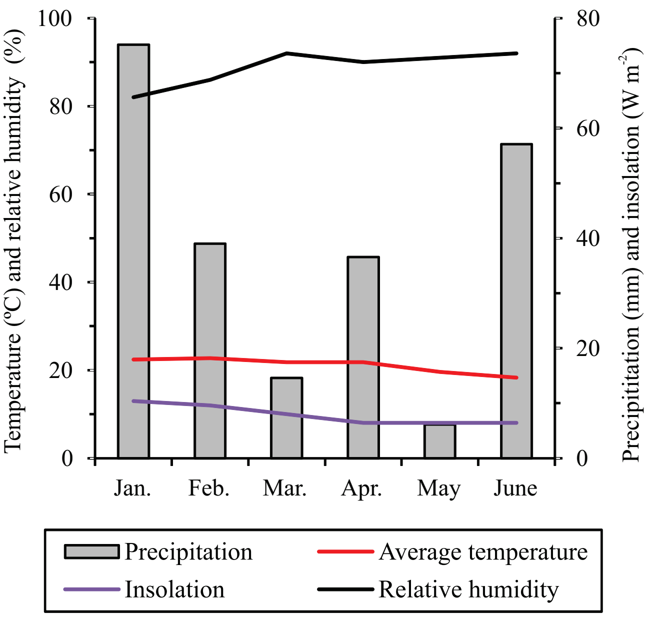

Figure 1. Climate data of precipitation, relative humidity, insolation, and average temperature, during the experimental period obtained at the weather station of Universidade Estadual do Sudoeste da Bahia.

11 tunnels. The experimental design was completely randomized, with 12 replicates.

To control the treatments, the primary buds of the inflorescences were randomly selected one day before anthesis and covered with voile fabric. From the other primary flowers of the same cultivar that had been pre-selected the day before pollination, pollen grains were collected and stored in Petri dishes at room temperature.

On each day of the MCP treatments (first, second, third, fourth, and fifth day of flower anthesis), the pollen was meticulously transferred to the flower stigma using a synthetic brush. Subsequently, the flowers were re-bagged, which was how they were kept until the development and maturation of the flower receptacle. For the NP treatments, on the day of each treatment, the flowers were uncovered to be exposed to natural pollination. In the case of SP, the buds were bagged until the development and maturation of the flower receptacle.

The fruits produced as a result of the treatments were harvested and quantified when 75% of their external surface showed a red to fully-ripe coloration. On the same day as that of the harvest, the analyses were conducted at the Laboratory of Biodiversity of the Semiarid Landscape at Universidade Estadual do Sudoeste da Bahia.

Fruit biometry was determined based on the following parameters: fresh mass (g); longitudinal length (mm); and equatorial diameter (mm), measured at the median region of the fruits.

The fruits were classified according to the degree of deformation (DOD), using four categories recommended by Malagodi-Braga (2002), regarding shape, well-formed (without deformation); regular (slight deformation); deformed (medium deformation); and very deformed (severe deformation) (Malagodi-Braga, 2002).

Afterwards, the fruits were separated to determine the fertilization rate of the achenes according to Thompson (1971). For this, the achenes were extracted with tweezers and placed in containers with water to evaluate their flotation capacity: the viable achenes were those that sunk and the non-viable ones were those that floated.

The percentage of fertilized achenes (PFA) was calculated using the formula: PFA = (number of fertilized achenes/total number of achenes per fruit) x 100.

The collected data were subjected to the normality and homogeneity tests, followed by the analysis of variance. The means of the treatments were compared by the Scott-Knott test, at 5% probability, using the SISVAR statistical software (Ferreira, 2011). In addition, the Chi-square test, with a probability lower or equal to 5%, was carried out using the PAST software (Hammer et al., 2001).

In order to elucidate the relationship between the evaluated variables and the different treatments, the data were also subjected to the principal component analysis (PCA) using the XLSTAT, version 19.2.2, software (Addinsoft, 2019).

Results and Discussion

Direct observations of flower longevity revealed that the corolla opens at various times of the day and that the flowers persist until the fifth day, showing distinctive characteristics (Figure 2 A–F). According to Barbosa (2009), the stigmas of the flowers of the Aroma strawberry cultivar become progressively receptive to pollination, as follows: only the base on the first day, both the base and sides on the second day, and all of it on the third day. At pre-anthesis, the anthers exhibited a yellow hue, darkening as the

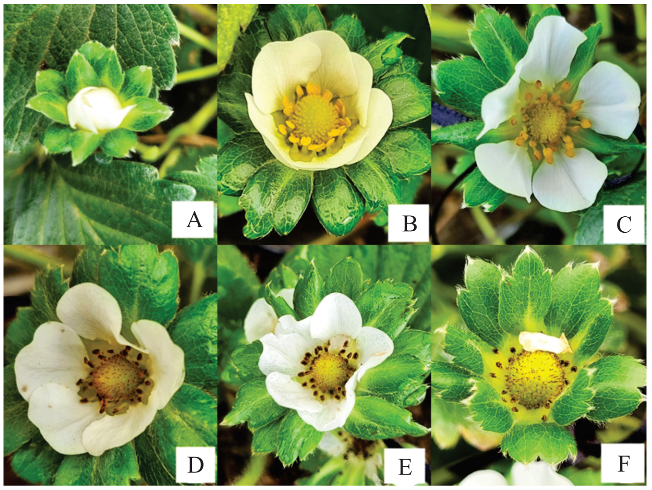

Figure 2. Flower of the San Andreas strawberry (*Fragaria x ananassa*) cultivar as a bud (A) and on the first (B), second (C), third (D), fourth (E), and fifth (F) day after opening. Photos by Priscila Silva Miranda.

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pollen grains were released (Figure 2 B–F). As the days progressed, in addition to the anthers darkening, the stigmas remained receptive until the fifth day when the beginning of the development of the flower receptacle became noticeable (Figure 2 F). Therefore, anther dehiscence is longitudinal and initiates shortly after corolla opening, with complete pollen exposure on the second day.

Malagodi-Braga (2002) observed a diverse flower longevity for cultivars Sweet Charlie and Oso Grande, whose flowers remained open for up to six days in the winter and spring seasons, i.e., from May to October. For cultivar Aromas, Barbosa & Orth (2020) recently reported a longevity of approximately six days in one year. These results suggest variability in the longevity of strawberry flowers among the different cultivars evaluated to date.

Concerning the physical characteristics of the fruits, the weight and length variables showed the lowest averages in the MCP5, NP5, and SP treatments during summer (Figure 3 A). In the fall, lower values were recorded in NP5 and SP (Figure 3 B).

Regarding treatment days, the values obtained in MCP and NP between the first and fourth days are aligned with the average of 12.96 g per fruit found by Farnezi et al. (2020) for the San Andreas cultivar. However, on the fifth day, fruits from the MCP and NP treatments showed a lower average weight. Moreover, length and diameter did not differ until the fourth day as a function of the MCP and NP treatments when compared with SP.

Regarding diameter, the variable did not differ significantly between pollination treatments, except in SP, in the first evaluation period, but showed lower averages in NP5 and SP in the second period. Contrastingly, Abrol et al. (2019) reported larger fruits (diameter and length) under NP than under SP. These results are an indicative that the degrees of dependence on specific pollination processes may differ between cultivars.

The relative frequency of fruits with different DODs varied between evaluation periods and treatments (χ^2= 113.44, $p<0.05$; χ^2=67.83, $p<0.05$; and n=12, respectively). In the first period, most of the fruits were either well-formed or with up to 30% deformation until the fourth day of MCP and the third day of NP (Figure 4 A). In the second period, most fruits with the lowest percentage of deformity were observed in the first two days of MCP and in the first three days of NP (Figure 4 B). In both study periods, only treatments MCP1, MCP2, NP1, NP2, and NP3 did not present fruits with a severe deformation. Furthermore, SP resulted in fruits with 100% deformation in the first period and a 70% severe deformation in the second.

In the literature, Barbosa & Orth (2020) found a higher percentage of well-formed fruits (73%) from the third day of flower anthesis in the Aromas cultivar. For the San Andreas cultivar, Silva et al.

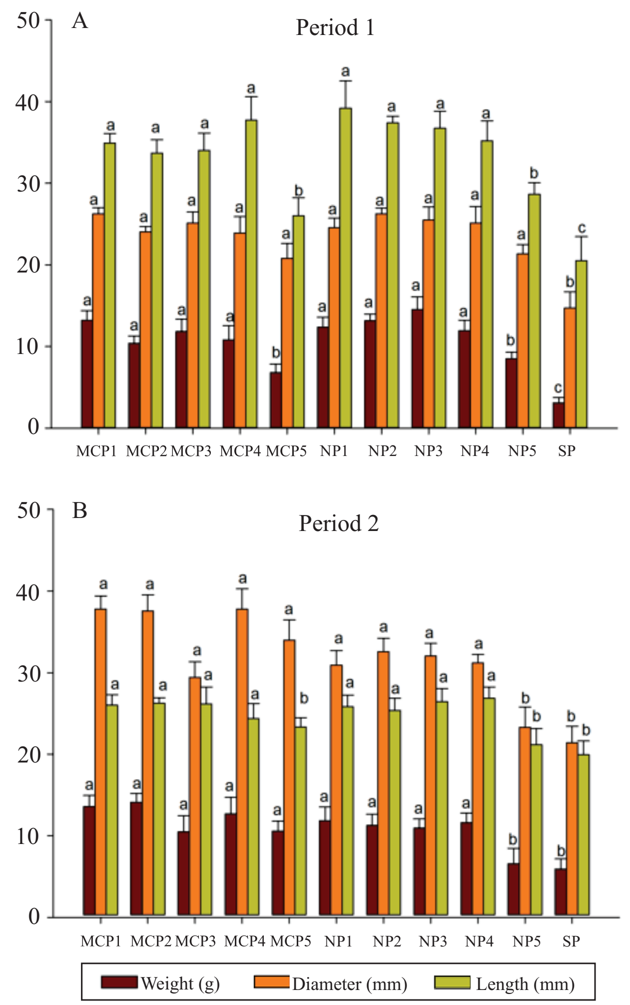

Figure 3. Physical analyses of the San Andreas strawberry cultivar, showing the weight, diameter, and length of the fruits resulting from 11 pollination treatments performed in period 1, during the summer (A), and in period 2, during the fall (B) of 2022. Treatments: MCP1 to MCP5, manual pollination on the first to fifth day of anthesis; NP1 to NP5, natural pollination on the first to fifth day of anthesis; and SP, self-pollination.

(2020) obtained well-formed fruits on the first day of anthesis. Contrastingly, in the present study, pollination treatments after the third day caused a decrease of 10% in the percentages of well-formed fruits, suggesting that some stigmas were no longer receptive or were not fertilized, resulting in lower fertilization rates and in increased fruit deformation rates. In addition, the receptivity at the stigmas of the strawberry flowers was varied and gradual, potentially leading to poorly formed fruits depending on pollination day. According to Klatt et al. (2014), evenly distributing pollen in flowers is important to ensure the fertilization of the stigmas for a uniform growth of the flower receptacle and well-formed fruits.

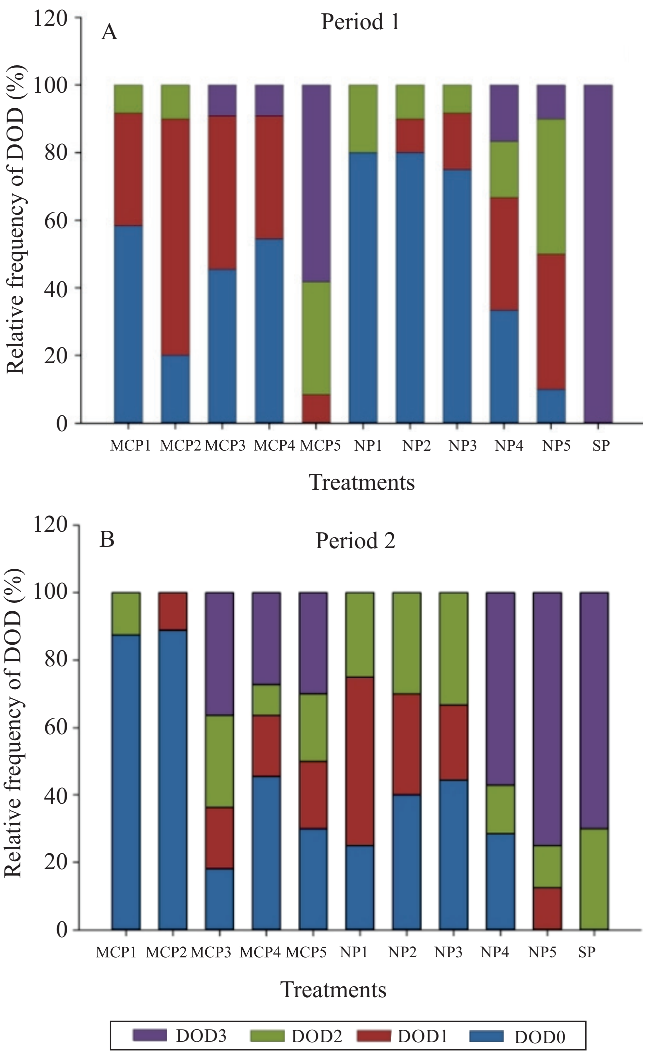

Figure 4. Percentage of fruits of the San Andreas strawberry cultivar with different degrees of deformation (DOD) caused by 11 treatments in period 1, in the summer (A), and in period 2, in the fall (B) of 2022. DOD0, well-formed fruits; DOD1, deformation up to 30%; DOD2, deformation from 30–60%; and DOD3, deformation above 60% (PBMH & PIMO, 2009). Treatments: MCP1 to MCP5, manual pollination on the first to fifth day of anthesis; NP1 to NP5, natural pollination on the first to fifth day of anthesis; and SP, self-pollination.

As to the PFA, in the first period, the average was approximately 31% in treatment MCP5 (Fc=14.04, $p<0.05$), which was the lowest rate compared with those above 60% of the other treatments (Table 1). In the second period, the lowest averages were below 50% for treatments NP2, NP3, NP4, and NP5. PFA is an important variable since it indicates the effectiveness of pollination (Csukasi et al., 2011), being closely related to strawberry fruit formation and fresh mass (Klatt et al., 2014), considering that, when the ovules are not fertilized, the receptacles do not develop, leading to abnormal or small-sized fruits.

In the first period, the joint analysis of data using PCA indicated that the variables diameter and PFA accounted for the variation along principal component 1 (PC1) and explained 89.60% of the dissimilarity among treatments (Figure 5 A). Conversely, weight, length, and DOD were the attributes most associated with principal component 2 (PC2), explaining 5.58%

Table 1. Percentage of fertilized achenes of the San Andreas strawberry cultivar in two periods of 2022 under 11 treatments(1).

Treatment(2)	Percentage of fertilized achenes	
	Period 1 - summer	Period 2 - fall
MCP1	81.34a	74.84a
MCP2	84.56a	83.32a
MCP3	83.20a	62.44a
MCP4	84.93a	63.95a
MCP5	30.96b	59.30a
NP1	85.71a	59.39a
NP2	80.09a	46.63b
NP3	68.60a	50.53b
NP4	67.87a	45.97b
NP5	70.57a	38.06b
SP	ND(3)	ND(3)
CV (%)	16.82	29.72

(1)Means followed by equal letters, in the columns, do not differ by the Scott-Knott test, at 5% probability. (2)Treatments: MCP1 to MCP5, manual pollination on the first to fifth day of anthesis; NP1 to NP5, natural pollination on the first to fifth day of anthesis; and SP, self-pollination. (3)SP resulted in fruits with a high degree of deformation that were not compatible for this evaluation.

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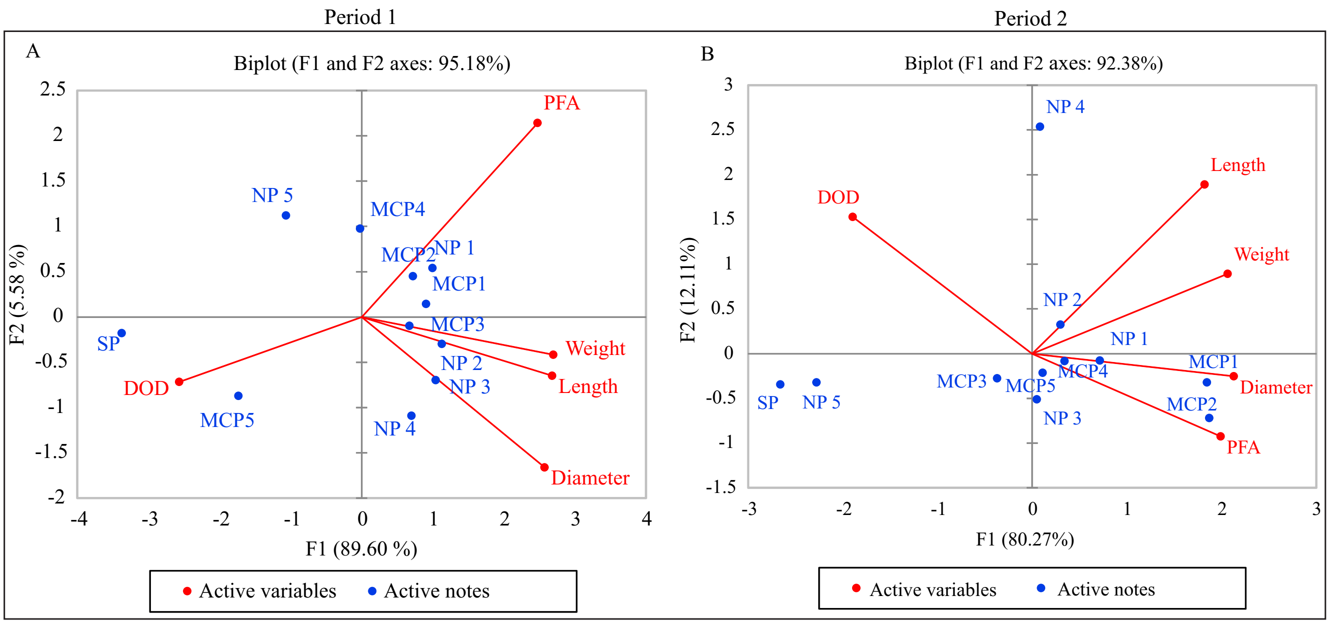

Figure 5. Ordination diagrams produced by the principal component analysis of the weight, diameter, length, degree of deformity (DOD), and percentage of fertilized achenes (PFA) of the fruits of the San Andreas strawberry cultivar under 11 treatments in period 1, in the summer (A), and in period 2, in the fall (B) of 2022. Treatments: MCP1 to MCP5, manual pollination on the first to fifth day of anthesis; NP1 to NP5, natural pollination on the first to fifth day of anthesis; and SP, self-pollination.

of the variation. In the second period, the data analysis revealed that along PC1, length, DOD, and PFA explained 80.27% of the heterogeneity among treatments (Figure 5 B). In contrast, diameter and weight expressed a 12.11% dissimilarity.

The scatter plot obtained through PCA suggests that the behavior of the treatments pollinated in the first days of anthesis is correlated with weight, diameter, length, and PFA. Furthermore, NP and MCP in the initial days of flower opening resulted in a more effective pollen distribution over the stigmas in the lateral and basal regions of the floral receptacle. Flowers pollinated in the early days exhibited a similar behavior, producing fruits without a severe deformation, indicating a complementary relationship between crop pollination and fruit formation (Klatt et al., 2014; Abrol et al., 2019). However, flowers pollinated on the fifth day and through SP showed a higher correlation with the DOD.

Conclusions

1. The flowers of the San Andreas strawberry (*Fragaria* x *ananassa*) cultivar show a prolonged longevity, remaining open until the fifth day of anthesis.

2. The first and second days of flower anthesis represent the optimal periods for both natural and manual-cross pollination in the San Andreas cultivar, resulting in fruits with a smaller degree of deformity.

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