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Hand-pollination in *Actinidia deliciosa* 'Hayward': how timing matters to improve fruit quality

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Short title: **Timing of hand pollination in kiwifruit**

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Abstract

Kiwifruit (*Actinidia deliciosa* 'Hayward') is a dioecious species where limited pollen transfer frequently constrains fruit quality, leading to rely on manual pollen applications. However, the optimal timing and frequency of delivery remain critical for maximizing efficiency. This study evaluated the effects of single (Trial A; days 1 to 7 of anthesis) and triple manual pollen application (Trial B; 3 g/L) on stigmatic pollen load, seed count, fruit weight, and fruit shape through a novel Fruit Shape Index (FSI). Applying a single dose of pollen involved higher viable pollen loads on stigmas during the first 4–5 days of anthesis. Pollen delivery within the first four days increased seed count per fruit, but maximum fruit weight was achieved only when pollen was applied within the first two days (48 h). Stigmatic fertile pollen adhesion and fruit quality in triple-application schedules similarly improved only when applied within the first four days. Comparing optimal treatments between trials revealed no significant differences, demonstrating that a single dose (3 g/L) within the first 48 h is sufficient to reach high commercial quality standards. Maximizing fruit weight and seed count did not automatically improve fruit shape symmetry. Hand-pollination logistics should prioritize delivering a single pollen dose within the first 48 h of flower opening. Subsequent or repeated manual applications beyond day four are ineffective, providing a clear strategy to optimize fruit quality while eliminating redundant labor and pollen costs.

Keywords: kiwifruit, stigma, fertility, crop productivity

Key Policy Highlights

- The effective window for manual pollination in kiwifruit (*Actinidia deliciosa* ‘Hayward’) extends up to 4–5 days post-anthesis.

- Fruit weight reaches its highest values when manual pollen application occurs within the first 48 hours after flower opening.
- Orchard management strategies should prioritize applying a single pollen dose during the first two days of anthesis, avoiding unnecessary repeated applications on older flowers.
- The proposed Fruit Shape Index provides an independent metric to evaluate fruit symmetry in quality assessments.

1. Introduction

Actinidia deliciosa 'Hayward' has become a major fruit crop in global markets (FAO, 2014; Satpal et al. 2021). As a dioecious species, its commercial productivity relies on the efficient transfer of fertile pollen from male to female flowers, which is a critical determinant of fruit set and quality (Costa et al. 1993; González et al. 1998). Several agronomic factors affect pollination success, including the female-to-male plant ratio and distribution (Goodwin et al. 2013; Meroi Arcerito et al. 2024), the use of managed pollinators such as *Apis mellifera* (Goodwin et al. 2013; Meroi Arcerito et al. 2021), and supplementary techniques such as hand-delivered pollen (Testolin and Ferguson 2009) or pruning and thinning techniques (Pescie and Strik 2004). However, several studies have reported pollination deficits in kiwifruit, leading to reduced fruit set, fewer seeds, and lower fruit weight that compromise market quality (e.g., Craig et al.1988; Goodwin and Perry 1992; González et al. 1998; Razeto et al. 2005). Beyond the congenital malformations established during floral bud development (e.g., fasciation) (Watson and Gould 1993), and the pre-anthesis factors that determine initial ovary size (Lai et al. 1990), fruit symmetry is further influenced by seed distribution. Thus, in non-fasciated kiwifruit, and following the

general patterns of berry-type fruits, localized pericarp growth is stimulated by the presence of seeds. Consequently, uneven seed set among locules leads to an asymmetrical fruit shape (Hopping 1976).

Despite the wide range of strategies aimed at enhancing pollination, sole reliance on insect pollination may be insufficient to achieve commercially desirable fruit set (Craig et al. 1988; Goodwin and Perry 1992), as weather conditions and colony strength strongly influence the role of *A. mellifera* in the pollination service (Evans et al. 2026). Consequently, hand-pollination has become a common practice worldwide, particularly in orchards with poor pollinator activity, degraded landscapes, or during adverse climatic conditions, while the crop's short flowering period occurs (Corbet et al. 1982; Holcroft and Allan 1994; Mazzei et al. 2024; Yao et al. 2010). In summary, *A. mellifera* is an effective pollinator of kiwifruit in maximizing both fruit set and fruit quality (Delaplane and Mayer 2000; Goodwin et al. 2013; Meroi Arcerito et al. 2021; David et al. 2022), and despite a range of strategies to enhance insect pollination (e.g., Meroi Arcerito et al. 2021; 2024; Verellen et al. 2025), hand-delivered viable pollen remains a worldwide practice adopted mainly in Chile (Razeto et al. 2005), Spain (González et al. 1998), Italy (Testolin and Ferguson 2009), the United States (Abbate et al. 2023), New Zealand (Hopping and Hacking 1983), southern China (Huang 2016), Korea (Lim et al. 2014; Lee et al. 2019), and Argentina (Meroi Arcerito et al. 2024). Pollen can be delivered dry or in an aqueous suspension, and is typically applied to female flowers using specialized devices (Broussard and Pattemore 2023). Previous research has shown that hand-pollination increases fruit set and fruit quality, with higher seed numbers and fruit weights than natural pollination, highlighting its value in improving pollination under pollination deficits (González et al. 1998; Razeto et al. 2005; Huang 2016). In this scenario, a key biological factor determining reproductive success is stigmatic receptivity, defined as the ability of the stigma to support pollen adhesion and germination. The stigma is the first female structure

that pollen grains encounter, and its receptivity establishes the period during which effective pollination and germination can occur. In kiwifruit, information about the duration of stigmatic receptivity is scarce, but previous studies suggest that it declines markedly after the fourth day of anthesis (González et al. 1995; González et al. 1996). Such a decline may reduce pollen adhesion and subsequent fertilization, thereby limiting both fruit set and seed development (Yi et al. 2006). However, little is known about how the timing of pollen application across anthesis or the frequency of pollen delivery influences final fruit quality. *Actinidia* spp. have a short flowering period that varies by cultivar and weather conditions; for instance, *A. chinensis* female flowers remain receptive for about seven days (Broussard and Pattemore 2023). From an agronomic perspective, identifying the period of maximum pollen adherence to the stigma, enhancing germination chances, and understanding the consequences for fruit quality could help growers optimize kiwifruit productive capacity. Therefore, our goal was to evaluate fruit quality by comparing (i) the timing of a single, manual pollen application to female flowers on different days of anthesis (Trial A), and (ii) the effects of three multiple pollen applications to female flowers across different combinations of days during anthesis (Trial B), and whether this multiple application of fertile pollen has an additional positive impact on fruit quality compared to a single application. We hypothesize that fruit quality (fruit weight, seed count, and shape) is affected by the timing of pollen application, which is also dose-dependent, due to changes in the adherence of fertile pollen to stigmas during anthesis, where only one application might be enough to achieve maximum quality. These findings may contribute to the development of more effective manual pollination strategies for kiwifruit.

2. **Materials and Methods**

2.1. Study site and experimental design

Fieldwork was conducted at Santa Paula farm (38°10′06" S, 57°38′10" W). The experimental design consisted of two independent but complementary experiments of controlled manual pollination to compare the effects on pollen adhesion to the stigma and fruit quality: Trial A, to determine the optimal timing of a single hand-pollination during the different days of flower anthesis for improving the timing of pollen application and final fruit quality, and Trial B, to evaluate whether applying an increased manual pollen dose (i.e., three times) across the anthesis in different day-combinations could affect fruit quality. To exclude insect pollination, voile bags were used to isolate flowers before anthesis. Flower opening was monitored early in the morning by checking the marked flowers; those newly opened flowers were designated as “day 1” in the experiment. Thus, each flower was labeled by its first anthesis date and monitored individually. The floral “life span” was seven days. After a week, opened flowers usually exhibit colour changes and wilting in different organs and tissues (personal observations). Flowers of different plants were randomly assigned to each treatment, hand-pollinated once on different days after opening, and then bagged again to ensure that manual pollen delivery was the primary explanatory factor. The hand-pollination dose was standardized according to regular producer practices (same pollen dilution and application methods). A pollen solution (3 g/L in distilled water) was applied within 40 minutes after preparation to prevent inactivation from osmotic stress (Hopping and Simpson 1982; Matsumoto et al. 2007). Each application was performed using a hand-delivered sprayer (Giber, 1.5 L) and was sprayed from a distance of 10 cm from the flower to avoid tissue damage. Each spray liberates approximately 0.3 mL per flower. Given a concentration of 3 g/L, this volume contains 9×10^{-4} g of pollen. Based on a conversion factor of 2×10^{7} grains per gram of pure dry pollen (Hopping, 1981), each one-second spray delivers an estimated 1.8×10^{4} fertile pollen grains to the whole flower.

153

154 2.2. Trial A: Single pollen application at different anthesis days

155 This trial aimed to explore the optimum timing for applying a single dose of hand-delivered
156 pollen, with the counting of pollen grains on the stigma and fruit quality as response
157 variables. Thus, a total of nine plants were selected, each of which received all treatments
158 to account for inter-individual variation. The treatments consist of seven flowers per
159 treatment all of them in each plant to avoid any individual bias (D1 to D7, divided
160 according to the lifespan period of the flower, from the 1st day after flower opening to the
161 7th day before floral wilting, Fig. 1), the flowers (see Fig. 2 for a guide) were located in the
162 middle third of the main branches to prevent any bias in energetic allocation within the
163 plant. Then, five flowers of each treatment were allowed to fruit until harvest time, and two
164 flowers were collected for pollen count on the stigmas. These flowers were collected 24
165 hours after pollen delivery to favour pollen-tube development, thereby facilitating counting
166 and recognition under microscopy. The controls were two: control-Bagged, to test the
167 bagging effect and to determine if fertile pollen still reaches stigmas through the voile
168 (anemophilous pollination), and control-Open flowers as an indicator of the producer's
169 pollination management, which is made by several synergistic factors such as
170 anemophilous, entomophilous, and hand-delivered pollen according to the regular practice
171 in commercial crops of the region.

172

173 2.3. Trial B: Triple pollen application at different day combinations

174 This trial aimed to assess whether administering three doses during anthesis improves the
175 number of pollen grains (both fertile and infertile) on stigmas and fruit quality (# of seeds,
176 fruit weight, and shape), and whether this effect depends on application timing, treated as
177 an explanatory variable. In addition, by using pollen count as the response variable, we

were able to explore whether the observed pattern depends on differential adherence of pollen to the stigma as the flower ages, or not. Thus, a total of seven plants were selected, each containing all treatments, which consisted of ten flowers per treatment, following the Trial A selection for branches criterion. In those ten flowers, seven were followed until the fruits reached harvest time, and the last three were collected 24 hours after pollen delivery for stigma inspection under a microscope. Each combination of application of each of the three doses of fertile pollen by hand-delivered technique across different days during anthesis constitutes a different treatment; thus, treatment 1 (T1) involved an application of pollen doses on days 2, 4, and 6, treatment 2 (T2) involved days 2, 3, and 4, and Treatment 3 (T3) involved days 4, 5, and 6. The blooming life span (days 1 and 7) was not considered because we aim to establish a potential strategy of delivering pollen in a productive matrix where such extremes of flower age are difficult to select for manual pollen application. Thus, the selected days belong to the more plausible scenarios for the commercial crop, according to producers and personal experiences in previous seasons. We also set up two controls, as in Trial A.

2.4. Pollen counting

In flowers collected 24 h after pollen delivery for each treatment and trial, we counted fertile and infertile pollen grains on four stigmas per flower to estimate how much of each pollen type adhered to them. We first calculated the mean number of grains per stigma and then multiplied this value by 39, the average number of stigmas per flower, to obtain a standardized estimate of each pollen type per flower. The collected stigmas were placed in 10% chlorinated water for 5 minutes to induce elasticity and transparency in the tissue. Stigmas were washed in distilled water in a Petri plate, then stained with lactophenol stain, and then prepared by a squash

technique to observe under a microscope (40x), both fertile pollen (only the well-stained and with regular form) from functional male flowers and infertile pollen (almost not stained and/or with irregular forms), mostly from functional female flowers (Figure 3).

2.5. Fruit quality assessment

Once fruit reached the harvest time according producers practice (about six months after blooming), in May of 2024, we collected the fruits per treatment and trial to assess our response variables considered as proxies of fruit quality: registering fruit weight (g) using a precision balance, then # of seeds that were counted according to standards (Meroi Arcerito et al. 2021; Meroi Arcerito et al. 2024), and a novel index of shape (see below) was calculated aiming to account for commercial standards.

2.6. Fruit Shape Index

Unlike previous studies, where the shape index is usually influenced by fruit length (which in our data is correlated with fruit size), we aimed to avoid this correlation. Instead, we focused on identifying forms that producers consider undesirable, either because they affect consumer choice or interfere with mechanical grading systems used to sort fruits by size. We therefore propose a new index based on the ratio of maximum width to minimum width (maximum width/minimum width). This fruit shape index evaluates symmetry only in the equatorial (sagittal) plane and indicates whether the fruit is equatorially flattened. When the ratio is close to 1, the fruit is more rounded in the equatorial plane (desirable shape for growers) and less flattened than when the ratio deviates from 1.

2.7. Statistical analysis

The obtained data, either for # of pollen grains on the stigma or fruit quality (# of seeds, fruit weight, and shape), were modelled through generalized linear mixed models (GLMM) using R 4.3.3 (R Core Team 2021). The variable plant was included as a random factor in the models. To represent the estimated values of each selected model, we used plots made with the plot (R Core Team 2021) and ggplot2 (Wickham 2016) functions. We used the lme4 package (Bates et al. 2015) to conduct the GLMM and the fitdistrplus package (Delignette-Muller and Dutang 2015) to fit the best probability distribution for each analysed variable. Additionally, we employed the Maximum Likelihood Test to select the best-fitting model. The assumptions of the linear regression with binomial family were tested using the DHARMa package (Hartig 2021). In Trial A, the # of fertile and infertile pollen grains per stigma was modelled using a negative binomial GLMM to account for overdispersion. For fruit quality, seed count was log-transformed and analysed using a linear mixed model (LMM). Fruit weight followed a normal distribution; thus, an LMM was the best model fit, and Tukey-adjusted pairwise comparisons were performed. For the Fruit Shape index (FSI), the best model fit was a Gamma distribution in a GLMM.

In Trial B, for # of pollen grains on the stigma per flower as an estimator of stigma–pollen adhesion to stigma, the best model was a negative binomial model without a random effect. For fruit quality, the best-fitting model for the number of seeds was a negative binomial model. For fruit weight, the best-fitting model had a normal distribution. For the FSI, the best-fitting model was a Gamma distribution in a GLMM. In the comparison between Trial A and B, Plant was included as a random effect in all models to account for the data structure, since different individuals of the same crop were involved in both trials. For fertile pollen count, the data were analysed using a GLMM with a negative binomial distribution; this model incorporated a dispersion formula based on the Trial to account for heteroscedasticity between experimental groups. For the number of seeds, the best fit was obtained with a GLMM using a negative binomial distribution. Finally, for fruit weight, an

LMM was applied to the log-transformed response variable.

Comparison between Trial A and B

For this comparison, we selected the three variables that showed the best signal towards an indication of fruit quality in both trials: the amount of fertile pollen on the stigma, seed number per fruit, and fruit weight. To explore the best application strategy and recommend a fertile pollen dosage to growers, we selected the best treatment from Trials A and B and compared these variables

3. **Results**

3.1. Trial A: Single pollen application at different anthesis days

3.1.1. Timing in stigma-pollen adhesion

There were significant differences between experimental treatments (i.e., day after flower opening to deliver the pollen only once; $p = 0.0006$; Fig. 4a) in the # of fertile pollen grains per flower. The highest deposition was observed at D4 (1402 ± 281 grains) and D1 (1004 ± 207 grains). Conversely, the lowest pollen counts were found in control-bagged (396 ± 84 grains) and at D6 (493 ± 105 grains). From D5 onward, a general decreasing trend in fertile pollen adherence was observed (Table 1).

Regarding infertile pollen, significant differences were found in the adherence of pollen on stigmas across the different days of anthesis ($p = 3.41 \times 10^{-6}$; Fig. 4a). Infertile pollen attachment peaked at day 4 (D4) with 2934 ± 580 grains, a count significantly higher than those recorded at D3 ($p = 0.025$), D6 ($p = 0.008$), and the control-Bagged ($p < 0.001$). After the D4 peak, pollen adherence declined steadily toward the end of the anthesis

277 period (Table 1).

278 3.1.2. Fruit quality

279 *Number of seeds*

280 The number of seeds varied with the age of the flower at which a single dose of pollen was
281 applied ($p = 1.0 \times 10^{-6}$). The highest number of seeds was recorded for treatment D1 but
282 was not significantly different from D2, D3, and D4 (see Fig. 4b caption). Treatments from
283 D5 onwards (i.e., D5 to D7) and the controls (Open and Bagged flowers) showed
284 decreasing seed counts (Table 1, Fig. 4b).

285

286 *Fruit weight*

287 Fruit weight was greater in flowers from D1 and D2 treatments compared to the flowers
288 sprayed on the following days (i.e., D4 to D7; Fig. 4c) and the control-Bagged flowers (all p
289 ≤ 0.0001). Fruit weight in treatments D4 to D7 was not different from the control-Open
290 flowers ($p = 0.2913$) (Table 1, Fig. 4c).

291

292 *Fruit Shape Index*

293 The correlation between sagittal length and weight was $R = 0.701$ (Spearman). The fruit
294 shape index (FSI) showed a weak correlation with fruit weight ($r = 0.20$, Pearson). The FSI
295 differed across pollination treatments ($p < 0.001$) (Table 1) but was similar among
296 treatments (from 1.08 to 1.11; Fig. 4d). Overall, the control-Open flowers treatment yielded
297 the most symmetrical FSI measures among all treatments.

298

299 3.2. Trial B: Triple pollen application at different day combinations

300 3.2.1. Timing of multiple doses of pollen in stigma-pollen adhesion

301 The main differences for the amount of fertile pollen per flower were registered between
302 T2 and control-Bagged flowers ($p < 0.0001$), where estimated values showed that T2 had
303 8.4 times more fertile pollen grains than the control-bagged treatment (Fig. 5a). Control-
304 Open flowers also showed significantly higher deposition than the control-Bagged
305 treatment ($p = 0.003$), with 4.7 times more fertile pollen grains (Table 2).

306 Regarding infertile pollen, the control-Open treatment exhibited the highest number of
307 grains per flower, which was significantly higher than that of T3 ($p = 0.001$) and control-
308 Bagged flowers ($p = 0.0003$) (Table 2, Fig. 5a).

309

310 *3.2.2.* Fruit quality

311 *Number of seeds*

312 The model shows that Treatment 2 (T2) and Treatment 1 (T1) yielded more seeds than the
313 other treatments (Table 2), and there were no differences between T1 and T2 ($p = 0.59$).
314 There was a significant number of aborted fruits in the control-Bagged treatment; only
315 three fruits reached harvest season, avoiding being part of the statistical analysis (Fig. 5b).

316

317 *Fruit weight*

318 Treatment significantly influenced fruit weight ($p = 0.002$). Results indicated that T2
319 showed higher values of fruit weight (g), reaching 108.9 ± 2.59 g of estimated mean,
320 followed by T1 (99.2 ± 2.50 g), control-Open flowers (98.2 ± 3.16 g), and T3 (95.1 ± 2.72 g)
321 (Table 2, Fig.5c).

322

Fruit Shape Index

The Fruit Shape Index (FSI) did not show a significant effect among treatments ($\chi^2 = 3.13$, df = 3, p = 0.372) (Table 2). The ratio between maximum and minimum width remained consistent across all groups, with values ranging from 1.09 ± 0.06 (control-Open flowers) to 1.13 ± 0.08 (T1).

3.3. Comparison between the best treatments of Trials A and B

When analysing both Trials A and B, selecting the best treatment of each of them and comparing them together, we did not register any significant differences among treatments in any of the three variables selected. Specifically, the number of fertile pollen grains on the stigma per flower showed no significant difference (p = 0.779), nor in the total number of seeds per fruit (p = 0.468), nor in fruit weight (p = 0.775) (Figure 6).

4. **Discussion**

In this study, we sought to determine the optimal timing during the 7-day anthesis period for applying a single pollen dose to improve kiwifruit production. We also gathered detailed information to help maximize yields when additional pollen applications are used, responding to growers' needs for strategies that allow two or three manual applications on different days to guarantee pollination of most opened flowers. Our results showed that applying fertile pollen after the fifth day does not improve fruit weight and might result in an economically detrimental increase in production costs. In addition, no additional fertile pollen was registered on stigmas when the triple dosage was applied, suggesting that with only one application during the first two days of anthesis, the stigmas reached their load capacity and no more fertile pollen adhered to the stigma even when the dose was

repeated after this initial window. Thus, we do not recommend applying pollen more than once at 3 g/L. Conversely, Hopping and Jerram (1980) suggested that a single application of 2 g/L or even 1 g/L might be enough to reach high-quality standards in kiwifruit, but these lower concentrations have yet to be tested in this region.

The results may be explained first by differences in stigmatic receptivity, and then by decreased adhesion of fertile pollen to stigmas. In general, pollen grain capture depends on the physical and adhesive properties of the stigmatic surface, including papillar cell walls and surface composition (Heslop-Harrison and Shivanna 1977). Detailed anatomical studies in kiwifruit showed that 24 h after pollination, pollen tubes had reached the stylar canal and the transmitting tissue became disorganised (Hopping and Jerram 1979). Pollen-tube growth and guidance through the style are even more sensitive to pistil aging, as they rely on the structural and molecular integrity of the extracellular matrix (including arabinogalactan proteins and pectin), which progressively degrades during flower senescence (e.g., Losada and Herrero 2012). Additionally, the chemotropic guidance of pollen tubes toward the ovule (Higashiyama and Takeuchi 2015), mediated by signals from the inner ovular tissues, also deteriorates after pollen tube growth in *Actinidia deliciosa* (González et al. 1996), where, after the fourth day of anthesis, stigma receptivity declines (González et al. 1995). Therefore, our results reinforce the concept of a gradient in functional loss within the pistil as the flower ages, where the various processes related to the capacity for pollen germination, pollen-tube guidance, or fecundation itself might have begun to decline before the isolated stigma's ability to capture pollen grains was compromised in *Actinidia deliciosa*.

Pollen application and management might involve dry or wet hand-pollen delivery, typically applied in two or more passes through the orchard to ensure coverage of the greatest number of open flowers (growers' personal communication). Some regional practices are aligned with Tacconi and Michelotti's (2018) recommendations, where a single pass after a

fifth day can be effective in Italian orchards. The flowers in our experimental sampling reached the “petal fall” stage from the fifth day onward (see Figure 2), an age when applying a single dose of pollen had no impact on fruit quality in our results. Thus, considering our trials, applying more than once and after the 5th day of anthesis is not recommended.

Pollen-tube competition might reduce seed set at high pollen loads (e.g. Stephenson 1981; Burd 1994; Aizen and Harder 2007); for example, flowers can receive more pollen than the “optimum doses” for ovule fertilization, which could be mediated by repeated hand pollinations (Hopping 1990). This has a detrimental effect not only in wasting fertile pollen, but also negatively impacting fertilization and fruit quality (e.g. Ashman et al. 2004; Rollin and Garibaldi 2019). Our results of multiple pollen applications showed neither detrimental nor beneficial effects on kiwifruit quality. Overall, the timing of anthesis was the main driver of the quality obtained.

From a commercial perspective, this FSI is a critical attribute; flattened fruits are often penalized by consumers and can present logistical challenges, such as complicating mechanical sizing and packaging during post-harvest handling. Our results demonstrate that maximizing fruit weight and seed set through supplemental hand-pollination does not necessarily translate into improved fruit symmetry. In addition, regarding the proposed new shape index, which is based solely on width measurements, we recommend using this width-based ratio, here called the Fruit Shape Index (FSI), as a fruit quality index rather than relying on sagittal measurements. The low correlation between fruit weight and our FSI ($r = 0.2$) indicates that this ratio provides independent information about fruit symmetry, in contrast to sagittal dimensions, which are redundant given their high correlation with weight, as previously reported (Meroi Arcerito et al. 2021, 2024). In fact, this new FSI is likely governed by the uniformity of seed distribution within the locules rather than the total mass of the fruit. The Fruit Shape Index (FSI), calculated as the maximum-to-minimum

fruit width ratio, showed no clear tendency across treatments in both trials.

Our results contribute to the broader discussion on kiwifruit productivity, particularly regarding the ongoing debate about how to contribute to optimizing the pollination process under a commercial matrix (e.g., Goodwin et al. 2013; Sáez et al. 2019; Meroi Arcerito et al. 2021; David et al. 2022; Broussard and Pattemore 2023; Meroi Arcerito et al. 2024). The effectiveness of hand-delivered fertile pollen on female flowers depends on several factors; undoubtedly, the way to deliver the fertile pollen and the amount to apply is the centre of debate in this topic. Here, we aimed to shed light on this debate, seeking the best commercial success; thus, this work contributes to understanding the dose, application strategy, and timing of pollen application as key factors in the hand-pollination process.

5. Conclusions

- The quality of the fruit depends on the timing (i.e., age of the flower) when manual application of fertile pollen is performed. Hence, hand-pollination could be optimized by considering the number of days since anthesis begins.
- Every hand-delivery strategy (a single dose during flower anthesis) should maximize spraying fertile pollen on the first two days (up to four) after flower opening in the whole crop to increase fruit quality.
- We do not recommend manually delivering fertile pollen on flowers older than four days since the beginning of anthesis.
- Under these trial conditions, a single pollen dose (Trial A) was sufficient to reach high fruit quality standards. Increasing to multiple or triple applications (Trial B) did not improve pollination parameters or fruit quality, indicating that successive applications simply represent an inefficient use of fertile pollen.
- Fruit shape symmetry is a key quality attribute for producers, and our results

indicate that the timing of manual pollen application did not lead to a clear tendency to reduce flattened fruit.

- Our new Fruit Shape Index is recommended to be used in fruit quality studies instead of other indices, being also aligned with the morphological standards sought by producers.

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Disclosure statement

The authors declare that they have no financial or personal conflicts of interest that could have inappropriately influenced or biased the research reported in this manuscript.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Declaration of Generative AI in Scientific Writing

During the preparation of this manuscript, the author(s) used Grammarly in his free cost version in order to assist with language editing and grammatical refinement.

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Authors' Contributions

All authors revised and approved the final version of the manuscript as a collaborative effort. Specifically:

- **F.R. Meroi Arcerito:** Conceptualization, project administration, experimental design, field logistics, data analysis, original draft preparation, writing – review and editing, and overall study leadership as corresponding author.
- **C. Corti:** Conceptualization, field experimentation, data analysis, writing – original draft preparation, and manuscript revision.
- **F. Ramos:** Investigation (field labor) and experimental design development.
- **M.B. Lezcano:** Investigation (field experimentation) and methodology design refinement.
- **M.P. Mazzei:** Formal analysis and statistical evaluation.
- **G. Fernández de Landa:** methodology (pollen characterization and counting).
- **D.P. Porrini:** Investigation and field labor support.
- **L. Galetto:** Methodology and experimental design optimization to enhance statistical power, supervision, and critical revision.
- **M. Maggi:** Funding acquisition, grower liaison management, field support, and research team supervision.

Figures:

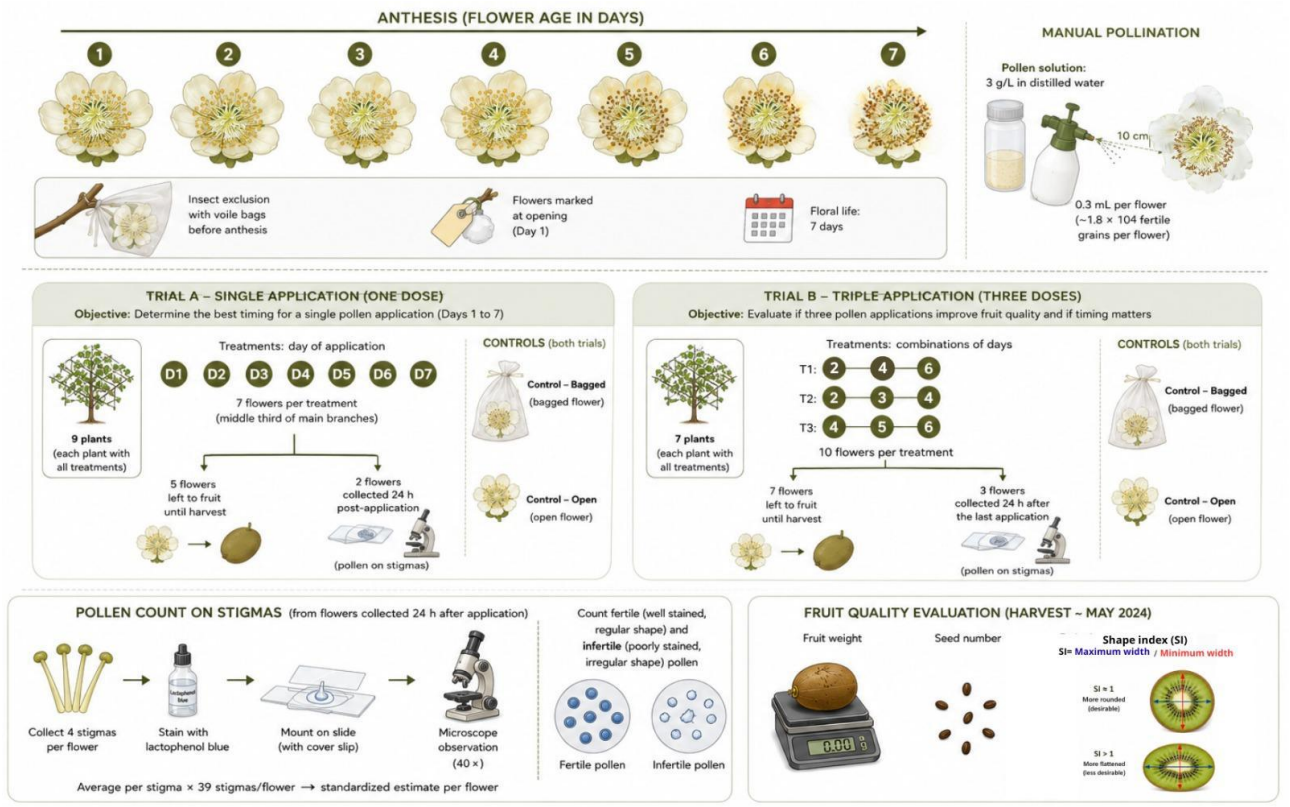


Figure 1. Summary of the methodological design.



Figure 2. Developmental stages (factor levels in our trials) and morphological changes of *A. deliciosa* flowers during anthesis.

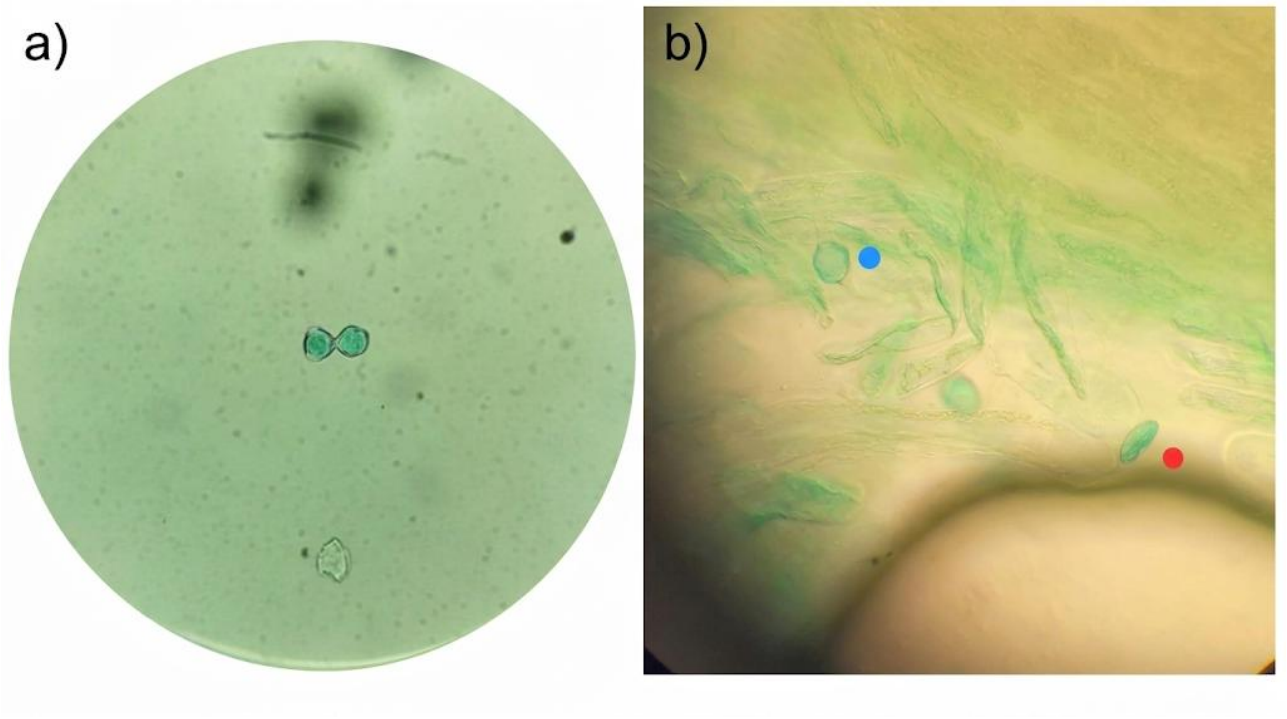


Figure 3. Pollen observation in *Actinidia deliciosa*. **(a)** Two fertile pollen grains in contact. **(b)** Fertile pollen grain with pollen tube germination (indicated by the blue dot). An infertile pollen grain marked by the red dot; note its flattened and "dehydrated" appearance compared to the fertile gamete. Photos by Germán Córdoba, a Bachelor's student in Biology and research intern in our research group.

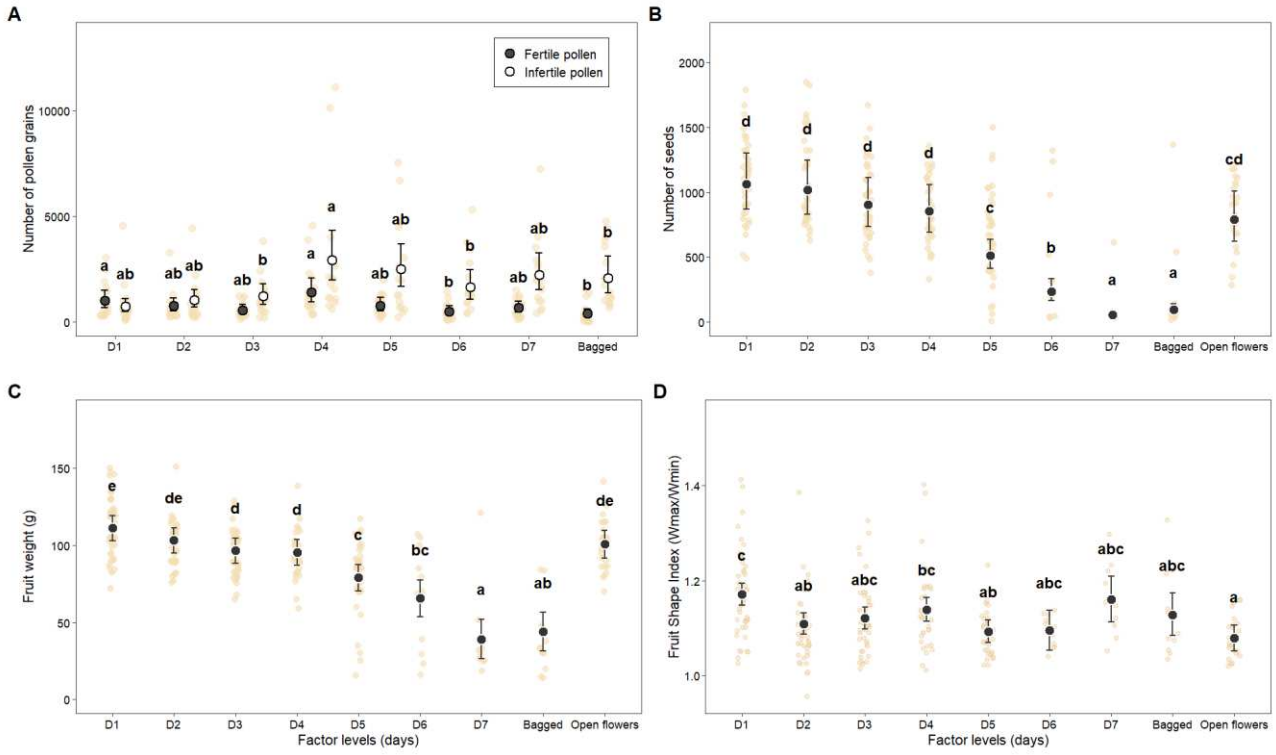
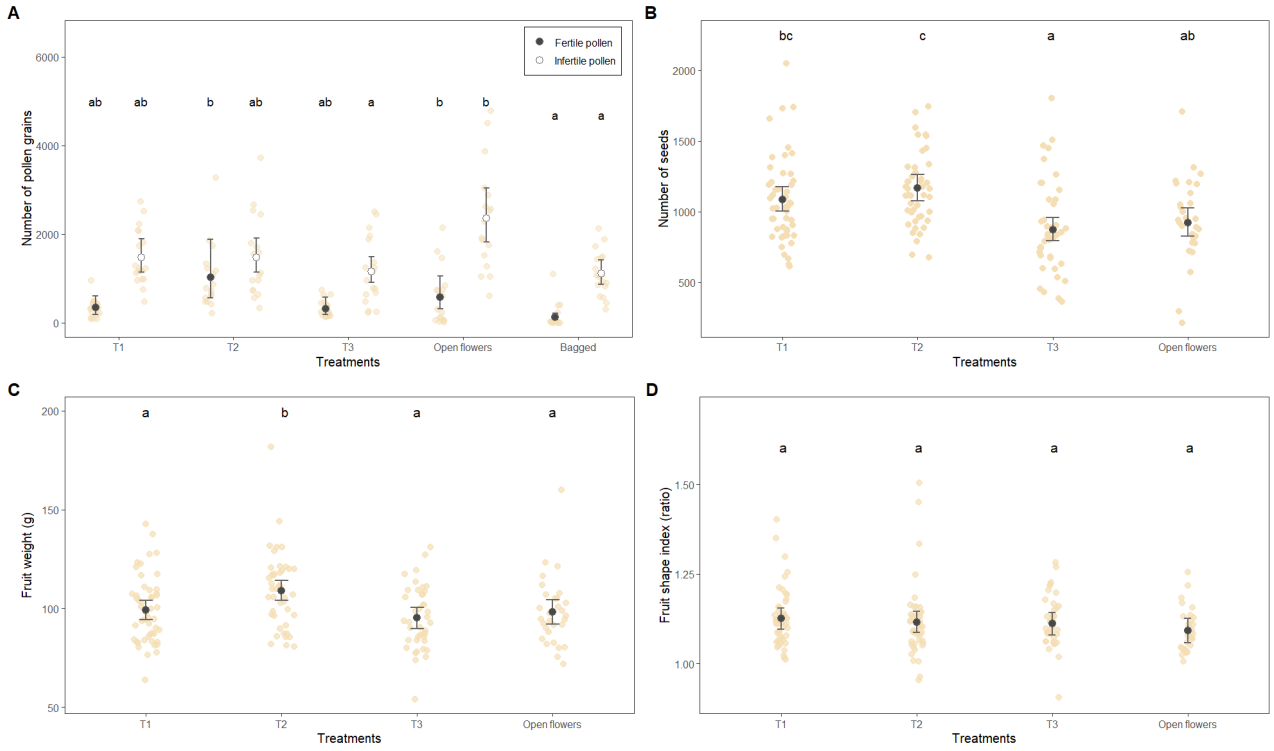


Figure 4. Trial A. *Effects of pollination timing on per-flower pollen adhesion to stigma and fruit traits*. The x-axis shows the different treatments corresponding to flower age in days during anthesis, and “Bagged” represents the control-Bagged, and “Open flowers” represents the control-Open flowers. The y-axis shows the response variables of the fit models. Light brown dots represent the estimated values, and black dots indicate the estimated marginal means derived from each model, with vertical bars representing the 95% confidence intervals. Different letters above the bars indicate significant differences between treatments according to post-hoc Tukey tests ($p < 0.05$). **A)** The y-axis shows the per-flower number of fertile (black dot) and infertile (white dot) pollen grains counted on stigmas. For fertile pollen, the highest adherence was recorded at D4 (1402 ± 281 grains), being significantly higher than D6 ($p = 0.0084$) and control-Bagged flowers ($p = 0.0004$).

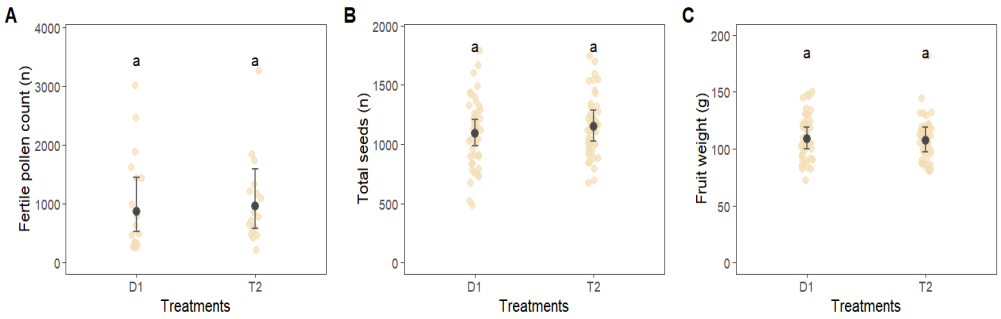
For infertile pollen, D4 showed the maximum attachment (2934 ± 580 grains), being significantly different from D3 ($p = 0.0253$), D6 ($p = 0.008$), and control-Bagged flowers ($p < 0.001$). **B)** The y-axis shows the number of seeds per fruit. The highest seed count was recorded on D1. Pairwise comparisons with D1 showed no significant differences for D2 ($p = 1.00$), D3 ($p = 0.94$), D4 ($p = 0.79$), and control-Open flowers ($p = 0.52$). Significant reductions in seed set were observed for D5 ($p = 2.33 \times 10^{-4}$), D6 ($p = 9.77 \times 10^{-9}$), D7 ($p = 1 \times 10^{-6}$), and control-Bagged ($p = 7.23\ 10^{-12}$) treatments. **C)** The y-axis shows the fruit weight (g). Maximum weight was achieved on D1 (111.2 ± 3.92 g) (LMM, $p = 0.0001$). Tukey's pairwise comparisons showed that D1 weight was not significantly different from D2 ($p = 0.5015$) or control-Open flowers ($p = 0.2913$). In contrast, D1 was significantly heavier than D3 ($p = 0.0059$) and D4 ($p = 0.0029$). While D3 and D4 showed no differences to each other ($p = 1.0000$), both were significantly heavier than D5 (D3 vs. D5, $p = 0.0009$; D4 vs. D5, $p = 0.0040$). Treatments D6, D7, and control-Bagged exhibited the lowest values, with no differences between D7 and control-Bagged ($p = 0.9995$), although D6 remained slightly heavier than D7 ($p = 0.0220$). **D)** The plot shows the Fruit Shape Index (FSI, Wmax/Wmin ratio) across pollination treatments. The highest FSI value was observed in D1, compared to D2 ($p = 0.0048$), D5 ($p = 0.0004$), and control-Open flowers ($p < 0.0001$). D4 also showed a significantly higher FSI than control-Open flowers ($p = 0.0477$). The treatments D3, D6, D7, and control-Bagged did not differ significantly from D1 ($p > 0.05$).



706

707 **Figure 5. Trial B:** *Effect of timing strategy on floral and fruit traits*. The x-axis represents
708 the treatments based on different combinations of flower age (days) during the deliveries
709 of manual pollination: Treatment 1 (T1: days 2, 4, and 6), Treatment 2 (T2: days 2, 3, and
710 4), and Treatment 3 (T3: days 4, 5, and 6). Thus, this trial involved three times more doses
711 of hand-pollen delivered as treatments in Trial A. Controls were abbreviated in the figure
712 as “Bagged” for control-Bagged and “Open flowers” for control-Open flowers. The y-axis
713 displays the response variables for each variable. Light brown dots represent the
714 estimated values, and black dots indicate the estimated marginal means derived from
715 each model, with vertical bars representing the 95% confidence intervals. Different letters
716 above the bars indicate significant differences between treatments according to post-hoc
717 Tukey tests ($p < 0.05$). **A)** The y-axis represents the number of infertile and fertile pollen
718 per flower for each treatment. There was more Infertile pollen in the control-Open flowers
719 compared to T3 ($p = 0.001$) and the control-Bagged ($p = 0.0003$), while showing a
720 marginal trend relative to T1 ($P = 0.0743$) and T2 ($p = 0.0843$). No significant differences
721 were found among manual pollination treatments (T1, T2, T3) and control-Bagged. In

Fertile pollen delivery, T2 and the control-Open flowers significantly outperformed the control-Bagged ($p < 0.0001$ and $p = 0.0029$, respectively), with estimated values 8.4 and 4.7 times higher, respectively. A marginal difference was observed between T2 and T3 ($p = 0.0506$). No significant differences were detected among T1, T3, and control-Open flowers. **B)** The Y-axis represents the number of seeds per fruit**.** T1 and T2 yielded the highest number of seeds per fruit, with no significant difference between them ($p = 0.58$). Both treatments significantly surpassed T3 (T1 vs. T3: $p = 0.0024$; T2 vs. T3: $p < 0.0001$), whereas only T2 was significantly higher than control-Open flowers ($p = 0.0027$); T1 exhibited a marginal trend relative to the latter ($p = 0.068$). Seed counts in T3 were comparable to control-Open flowers ($p = 0.88$). Control-Bagged was excluded from statistical assessment due to high fruit abortion rates, with only three fruits reaching maturity. **C)** The Y-axis represents fruit weight (g). Treatment 2 (T2) produced significantly heavier fruits (108.9 ± 2.59 g) than all other groups, including T1 (99.2 ± 2.50 g; $p = 0.0375$), T3 (95.1 ± 2.72 g; $p = 0.0017$), and control-Open flowers (98.2 ± 3.16 g; $p = 0.0453$). No significant differences were observed among T1, T3, and control-Open flowers ($p \geq 0.68$). **D)** The Y-axis shows the Fruit Shape Index. There were not significant differences detected ($p > 0.05$).



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745

746 **Figure 6.** *Direct comparison of the best treatments across the two trials (A and B)*. The x-
747 axis shows the treatments corresponding to the timing of hand-pollination in days after
748 anthesis: D1 (Trial A) represents a single application on day 1, and T2 (Trial B) involved
749 applications on days 2, 3, and 4. Trial B involved a threefold higher number of doses than
750 Trial A. Light brown dots represent individual observed values, black dots indicate the
751 estimated marginal means derived from the best-fit mixed model for each response
752 variable, with vertical bars representing the 95% confidence intervals. Different letters
753 indicate statistically significant differences between treatments (post-hoc Tukey test, $p <$
754 0.05). A) The y-axis represents the number of fertile pollen grains per flower. No significant
755 difference was detected between D1 and T2 ($p = 0.779$), indicating no significant
756 difference in the number of viable pollen grains on stigmas. B) The y-axis shows the total
757 seed number per fruit. No significant difference was observed between D1 and T2 ($p =$
758 0.468), thus, both pollination treatments resulted in comparable seed set. C) The y-axis
759 represents the fruit weight (g). No significant difference was detected between D1 and T2
760 ($p = 0.775$), indicating that fruit weight was similar under both treatments.

761

762

Table 1. Pairwise comparisons of pollen deposition and fruit quality traits

Trial A — Timing of a single pollen dose during anthesis

Comparison	Fertile pollen	Infertile pollen	No. seeds	Fruit weight (g)	FSI
Model	*Neg. Bin. GLMM*	*Neg. Bin. GLMM*	*LMM (log-transformed)*	*LMM*	*Gamma GLMM*
D1 vs D2	ns	ns	$p = 1.00$	ns	$p = 0.0048$
D1 vs D3	ns	ns	$p = 0.94$	$p = 0.0059$	ns
D1 vs D4	$p = 0.008$[a]	ns	$p = 0.79$	$p = 0.0029$	ns
D1 vs D5	ns	ns	$p = 2.33 \times 10^{-4}$	–	$p = 0.0004$
D1 vs D6	ns	ns	$p = 9.77 \times 10^{-9}$	–	ns
D1 vs D7	ns	ns	$p < 1 \times 10^{-6}$	–	ns
D1 vs Control-Open	–	–	$p = 0.52$	ns	$p < 0.0001$
D1 vs Control-Bagged	$p = 0.036$[b]	$p < 0.001$	$p = 7.23 \times 10^{-12}$	–	ns
D3 vs D4	–	–	–	ns	–
D3 vs D5	–	–	–	$p = 0.0009$	–
D4 vs D6	$p = 0.008$	$p = 0.025$[c]	–	–	$p = 0.0477$[d]
D4 vs Control-Bagged	$p = 0.0004$	$p < 0.001$	–	–	–
D5 vs D3	–	–	–	$p = 0.0009$	–
D5 vs D4	–	–	–	$p = 0.0040$	–
D6 vs Control-Bagged	–	$p = 0.008$[e]	–	ns	–
D6 vs D7	–	–	–	$p = 0.0220$	–
D7 vs Control-Bagged	–	–	–	ns	–
Control-Open vs Control-Bagged	–	–	–	–	–

ns = not significant ($p > 0.05$); – = comparison not applicable.

[a] *D4 showed significantly higher fertile pollen than D6 ($p = 0.008$).*

[b] *D1 differed from Control-Bagged ($p = 0.036$).*

[c] *D4 peak significantly higher than D3 for infertile pollen ($p = 0.025$).*

[d] *D4 FSI significantly higher than Control-Open ($p = 0.0477$).*

[e] *Decreasing trend toward D6 and Control-Bagged; D6 vs Control-Bagged ($p = 0.008$) for infertile pollen.*

Models: fertile pollen = Negative Binomial GLMM (plant as random effect); infertile pollen = Negative Binomial GLMM; seeds = LMM on log-transformed counts (plant as random effect); fruit weight = LMM with Tukey-adjusted comparisons (plant as random effect); FSI = Gamma GLMM.

Tabe 2. Trial B — Multiple pollen doses during anthesis

Comparison	Fertile pollen	Infertile pollen	No. seeds	Fruit weight (g)	FSI
Model	*Neg. Bin. (no random effect)*	*Neg. Bin. (no random effect)*	*Neg. Bin.*	*LMM*	*Gamma GLMM*
T1 vs T2	ns	ns (p = 0.074)	ns (p = 0.59)	p = 0.038	ns
T1 vs T3	ns	ns	p = 0.002	ns	ns
T1 vs Control-Open	ns	ns	p = 0.068	ns	ns
T1 vs Control-Bagged	–	–	–[f]	–	–
T2 vs T3	p = 0.051†	ns	p < 0.0001	p = 0.002	ns
T2 vs Control-Open	–	ns (p = 0.084)	p = 0.003	p = 0.045	ns
T2 vs Control-Bagged	p < 0.0001	–	–[f]	–	–
T3 vs Control-Open	ns	p = 0.001	ns (p = 0.882)	ns (p = 0.882)	ns
T3 vs Control-Bagged	–	p = 0.0003	–[f]	–	–
Control-Open vs Control-Bagged	p = 0.003	p = 0.0003	–[f]	–	–

ns = not significant (p > 0.05); – = comparison not applicable.

† Marginal difference between T2 and T3 (p = 0.051) at α = 0.05; T2 showed 3.2 more fertile pollen than T3.

[f] Control-Bagged produced nearly all aborted fruits (only 3 reached harvest); excluded from seed and fruit quality models.

Estimated means (± SE): T2 fruit weight = 108.9 ± 2.59 g; T1 = 99.2 ± 2.50 g; Control-Open = 98.2 ± 3.16 g; T3 = 95.1 ± 2.72 g.

Models: fertile pollen = Negative Binomial (no random effect); infertile pollen = Negative Binomial (no random effect); seeds = Negative Binomial; fruit weight = LMM; FSI = Gamma GLMM.