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Effective pollination period and its influence on fruit characteristics of 'Hayward' kiwifruit

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Key words: anthesis, fruit set, fruit trait, kiwifruit, pollination.

Abstract: Pollination is crucial for producing marketable kiwifruit, and increasing revenue of growers. The objectives of this research were to determine the effective pollination period (EPP) of 'Hayward' *(A. deliciosa* A. Chev. C.F. Liang & A.R. Ferguson) and to determine fruit characteristics in relation to the time of pollination. Hayward kiwifruit showed no significant decrement of fruit set and fruit weight within the 4-day and 2-day period, respectively, however, the mean weight of fruit was ≥ 85 g within 4-day. Fruit set was 100% when pollination was carried out during the first 3 days following anthesis. Fruit set decreased to 20.71% when flowers were pollinated 5 days after anthesis and were practically nil by 6 days after anthesis. Fruit weight and size were the highest on days 1-2 after anthesis and reduced for flowers pollinated 3-4 days after anthesis. The lowest fruit weight and seed weight and number were observed when pollination was done on day 5. Hayward kiwifruit showed no significant drop in fruit set within the 4-day period, and thus, appears to have an EPP equal 4 days after anthesis. Thus, efforts for producing good quality and of marketable size fruit should be concentrated within the first 4 days after anthesis.

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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1. Introduction

Hayward kiwifruit (*Actinidia deliciosa*) is the most widely grown *Actinidia* crops (Ferguson, 1990). This cultivar is chosen based on its large fruit production and long storage life. Optimal kiwifruit production is highly dependent on the level of pollination because insufficient pollination is known to lead to unsatisfactory fruit size, shape and uniformity; however, pollination of kiwifruit is impaired by the dioecious nature of the species (Pyke and Alspach, 1986).

Flower receptivity can be evaluated by determining the effective pollination period (EPP). EPP is defined as the number of days following anthesis during which pollination is effective in producing marketable fruit. Various factors may affect the EPP. It was shown that the EPP could be affected by temperature, flower quality, and chemical treatments (Sanzol and Herrero, 2001). Because of dioecious nature of kiwifruit, other factors should be in a favorable condition to determine of EPP in

this species. For example, male vines can be properly distributed, and proper timing of beehive placement can be achieved. In addition, determining the EPP of kiwifruit species/cultivars could allow growers to optimize supplemental pollen applications by applying only during the EPP, and thus, reduce costs.

Kiwifruit flowers are receptive for only a few days following anthesis where pollination can be successful leading to a good marketable fruit set. The EPP may be restricted by limitation in three main events along the reproductive process, stigma receptivity, pollen tube kinetics and ovule longevity (Sanzol and Herrero, 2001).

Estimation reports are available for the different cultivar of kiwifruit. There are variances among the reported duration of the EPP and stigma receptivity. According to Galimberti *et al.* (1987), the EPP of Hayward cultivar reported 3 days in Italy. The EPP for *A. deliciosa* 'Hayward' was determined to be 4 days by Gonzalez *et al.* (1995) in Spain. It was discovered that the duration of stigma receptivity closely fit the EPP, thus it appears that the EPP is limited by stigma receptivity. Sale (1981) reported that the pistillate flowers are receptive for 7-9 days after anthesis (DAA) in New Zealand. Goodwin (2000) also reported this longer period, where the relatively constant and high receptivity was displayed for the first 8 days before dropping. Goodwin *et al.* (2013) found stigma receptivity to be highest during the first 2 DAA in *A. chinensis* 'Hort16A'. They noted that pistillate flowers from *A. chinensis* dehisced their petals after 2 days while *A. deliciosa* typically hold their petals for 5 DAA. The EPP for *A. deliciosa* 'AU Fitzgerald' and *A. chinensis* 'AU Golden Sunshine' was determined to be 4 and ≥5 days respectively by Thompson (2014). In golden kiwifruit cultivars, not only fruit set but also fruit characteristic was affected by DAA pollination. Fruit size, weight, and seed number were reduced on day 5 after anthesis in 'AU Fitzgerald' (Thompson, 2014). Thompson (2014) found that 'AU Golden Sunshine' showed no significant drop in fruit set or size within the 5-day period. However, differences in fruit weight, fruit size index and seed number were found between 1-3 and 4-5 DAA by Brantley (2016).

Fruit set of 'Hayward' cultivar is influence by the time elapsed between anthesis and pollination. It seems that fruit traits in this cultivar may be affected by days after anthesis pollination too. There are some papers reporting contrasting results about the effective pollination period on fruit quantitative and qualitative traits in this cultivar. Therefore, the aims of this research were to evaluate the effective pollination period of 'Hayward' and to determine fruit characteristics in relation to the time of pollination.

2. Materials and Methods

Experimental design

This experiment was conducted using 10-year old vines of 'Hayward'. Kiwifruit vines were grown in an orchard located in Astara, Guilan province, Iran (38°22'N; longitude of 42°50'E), 10 m altitude, trained to a T-bar system with plants spaced of 4×6 m.

Treatment application

Effective pollinated period (EPP) was studied for 3 years. 'Hayward' flower buds were bagged on May 21, 2013; May 18, 2014, and May 23, 2015, using wax paper bags (10.2 × 26.2 cm). Flower buds were bagged 1 day before anthesis; still completely closed but showing some white from petal unfolding, identified as "Stage 57" in BBCH phenology system (Salineroa *et al*., 2009). Anthesis was the day the flower petals opened. The detailed characterization of environmental conditions during the experimental period is shown in Table 1.

Table 1 - Air temperature, relative humidity (RH), sunny hours, precipitation and mean daily transpiration (mm d^{-1}) during three months in 2013-15

Year	Month	Temperature (°C)					Evaporation (mm/d)	Relative humidity (%)	Precipitation (mm)	Sunny hours
		Mean	Max.	Min.	Max. Abs.	Min. Abs.				
2013	April	13.0	16.5	9.5	25.4	4.2	43.1	81	81.8	115.2
	May	17.0	21.7	12.3	27.2	4.8	114.4	76	29.4	236.4
	June	22.5	27.2	17.7	32.8	13.0	175.7	73	58.7	278.3
2014	April	11.1	15.3	6.9	24.8	0.4	68.7	79	95.6	172.7
	May	19.1	23.6	14.5	30.8	10.4	142.8	74	34.7	249.3
	June	23.5	27.9	18.6	32.2	14.8	175	74	15.6	269.4
2015	April	9.6	14.9	12.2	27.2	5.0	59.8	85	44.6	120.6
	May	13.9	19.8	16.9	24.2	7.8	106.9	81	23.5	189.3
	June	20.6	28.1	24.3	33.4	17.0	184.7	70	0.8	236.1

For every year, pollen was collected from staminate vines (Tomuri) one day before anthesis and dried on paper at room temperature. The pollen was then sieved using a fine mesh (0.26 mm) to remove dehisced anthers and other impurities. Bag-isolated pistillate flowers were hands pollinated with the dried pollen before being re-bagged. Twenty four flowers were hand-pollinated each day at 0, 1, 2, 3, 4, 5, 6 and 7 DAA using dried pollen. Uniform and single flower inflorescences were hands pollinated during the study. Flowers were re-bagged using newly labeled bags immediately following hand-pollination to prevent subsequent open pollination.

Data collection

Fruit set of all EPP treatments was determined when bags were removed three weeks later after pollination. For fruit trait analyses, they were harvested on November 7, 2015, when the value of Total Solid Soluble (TSS) detected was =6.2°Brix. Fruit length (L, mm), major width (W1, mm) and minor width (W2, mm), fruit density (g/ml), fresh weight (g), fruit volume (ml), fruit size index [(L+W1+W2) * 3^{-1})] seed number and seed weight (g) as quantity characteristics were measured (Brantley, 2016). To determine the average seed weight of each fruit, weight of 100-seed samples was determined thrice and the average of these values was used to calculate the total seed number for each fruit of each treatment (Goodwin *et al.,* 2013).

Statistical analysis

Combined analysis of three years fruit set data was performed on according to a completely randomized design. Data of fruit traits were analyzed as a completely randomized design. All significant means were separated, using Duncan (P≤0.05). The correlation between fruit weight with seed number and weight were calculated via the software SPSS 22.

3. Results

Fruit set after hand pollination was the highest, averaging 100% during the first 3 days following anthesis (Fig. 1). However, no significant differences were found between the days of treatment 1-4 (P≤0.05). Fruit set dropped down to 20.71% in the flower pollinated at 5 DAA. By 6 DAA, fruit set was practically nil. Thus, the EPP was limited to the first 4 DAA (Fig. 1).

Fruit weight and size (equivalent water volume) were the highest on day 1 (Table 2). A reduction trend was observed in flowers pollinated 2 DAA, however, did not differ from 1 DAA (P≤0.05). Fruit weight and size were reduced for flowers pollinated 3-4 DAA and the lowest value was observed on those pollinated 5 DAA (P≤0.05). Fruit density, fruit length, and fruit size index were the highest in the 1 DAA (Table 2) because the values were reduced on 2-4 DAA and the lowest value observed on day 5. Fruit major and minor width, and seed weight and the

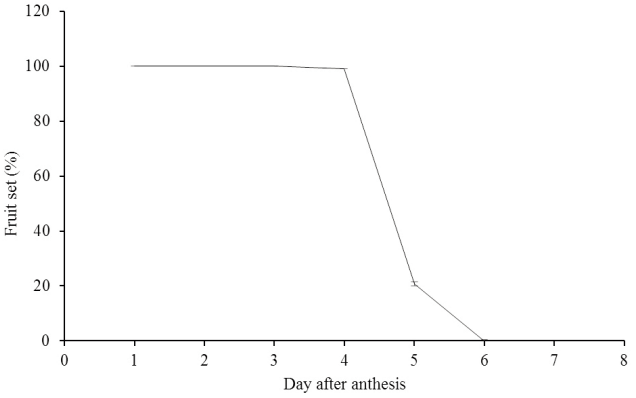

Fig. 1 - Effects of hand pollinating *Actinidia deliciosa* 'Hayward' flowers 1, 2, 3, 4, or 5 days after anthesis (DAA) on fruit set from 2013 to 2015. Values are the mean three years ± SE of three replicates.

Table 2 - Effects of hand pollinating Actinidia deliciosa 'Hayward' flowers 1, 2, 3, 4, or 5 days after anthesis (DAA) on fruit characteristics. Fruit were harvested 7 Nov. 2015

DAA	Weight (g)	Fruit volume (ml)	Fruit gravity (g/ml)	Fruit length (mm)	Major width (mm)	Minor width (mm)	Fruit size index	Seed weight per fruit (g)	Seed number per fruit	Seed weight (mg)
1	105.86 a	103.54 a	1.02 a	70.18 a	53.20 a	47.22 a	56.87 a	1.61 a	1218.97 a	1.33 a
2	95.10 ab	93.54 ab	1.016 b	64.86 b	52.72 a	46.35 a	54.64 b	1.56 a	1326.43 a	1.17 b
3	84.89 b	83.83 b	1.012 bc	63.18 b	50.42 ab	45.11 ab	52.91 b	1.56 a	1239.06 a	1.25 a
4	85.16 b	83.74 b	1.016 b	61.86 b	51.12 ab	45.07 ab	52.68 b	1.56 a	1226.35 a	1.27 a
5	71.73 c	71.10 c	1.008 c	56.44 c	49.37b	43.32 b	49.71 c	0.64 b	498.73 b	1.33 a

Each Values is the mean of three replicates with 15 fruits. Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test (P≤0.01).

number did not show significant reduction 1-4 DAA, however, the value was strongly reduced for flowers pollinated at 5 DAA (P≤0.05).

Correlation analyses showed a positive significant correlation between fruit size with seed number (r = 0.54**), and seed weight (r = 0.58***) (Table 3). The correlation between fruit weight with seed weight was higher than fruit weight with the seed number.

Table 3 - Correlation coefficient between fruit weight with seed number and weight in Actinidia deliciosa 'Hayward'

	Fruit weigh	Seed number	Seed weight
Fruit weight	1	0.54 **	0.58 ***
Seed number	0.54 **	1	0.95 ***
Seed weight	0.58 ***	0.95 ***	1

, * Correlation is significant at the 0.01 and 0.001 levels respectively.

4. Discussion and Conclusions

Effective pollination period is important in kiwifruit because successful pollination results in more seeds per fruit, and seed number directly correlates with fruit size and weight (Hopping, 1976). The results of this study for determination of EPP in 'Hayward' cultivar suggest that flowers should be pollinated within 4 DAA for the successful fruit set. EPP results of this study were similar to that founded by Gonzalez *et al.* (1995) for 'Hayward' in Spain. However, the greatest size, weight and fruit density occurred when flowers were pollinated within 1 DAA (2015). By extending the pollination period from 4 DAA to 5 DAA, a 79.29 % decrease in mean of fruit set was observed along the 3 years of observations.
A similar situation has been recorded under different cultural conditions for 'Hayward' (Gonzalez *et al*., 1995), AU Golden Sunshine' *(Actinidia chinensis*) and 'AU Fitzgerald' (*A. deliciosa*) (Thompson, 2014; Brantley, 2016).

EEP determined by the longevity of the ovules minus the time lag between pollination and fertilization (Sanzol and Herrero, 2001). EEP is affected by the growth rate of the pollen tube. The temperature has a clear effect on pollen tube growth rate (Hedhly *et al.,* 2005), and on flowering period of this study an optimal temperature for pollen tube growth (mean temperature 20.4 to 23.7°C) occurred during 3 years (Manandhar and Lawes, 1980). Due to the viability of ovules for the 7 days following anthesis in kiwifruit (Gonzalez *et al*., 1995), germination of pollen and pollen tube kinetics may be the limiting factor for EPP.

According to results of Gonzalez *et al*. (1995), the viability of ovules is 7 days in kiwifruit and an under optimal temperature, pollen tube reached the ovules 3 days after pollination, so that, there are 4 days between flower anthesis and fertilization. Thus, it appears stigma receptivity may not be the limiting factor for EPP, and under any condition, maximum EPP will be 4 days. However, Hopping and Jerram (1979) observed that the pollen tubes reached the style base and fertilized the ovules inside the ovary by 31 hours and 43 hours respectively. According to this result, there are more than 4 days between flower opening and pollination, and stigma receptivity may be the limiting factor. The difference in the pollen tube kinetics may be the result of pollen origin (Guerrero-Prieto *et al.,* 1985), nutritive stage of the flower (Nyomora *et al.,* 2000) or environmental conditions (Jefferies *et al.,* 1982).

EPP is short for kiwifruit. However, having a genetic component EPP with cultivars, it is affected by the nutritive state of the tree and weather alteration (Gonzalez *et al*., 1995), crop load (Crisosto *et al*., 1988; Buszard and Schwabe, 1995), temperature, flower quality, and chemical treatments (Sanzol and Herrero, 2001) and alternate bearing (Brantley, 2016).

In this study, fruit set averaged 99.8% for 4 DAA and decreased to 20.27% for day 5 in 'Hayward' cultivar along 3 years of studies. In the one-year study of Gonzalez *et al*. (1995) in this cultivar, fruit set averaged 80% after hand pollination during the first 4 DAA before declining to 36% on day 5 and then almost 0% by day 7. They attributed this to the loss of papillar integrity i.e. the unicellular papillae that cover the stigma began to rupture. They considered 80% fruit set to be successful pollination, and based on this, determined the EPP of 'Hayward' to be 4 DAA. They did not publish data for a seed count number or fruit size. Brantley (2016) reported averaging 98% fruit set for the first 4 DAA before declining to 81.5% for day 5 in 'AU Fitzgerald'. It seems plausible that this variance in fruit set was due to the alternate bearing tendencies of the species *A. deliciosa* (Morley-Bunker and Lyford, 1999), flower quality and pollen source (Brantley, 2016).

Presently, no research has been conducted on the effects of alternate bearing on pollination of kiwifruit flowers. However, EPP of *Malus domestica* L. Borkh. cv. Cox's Orange Pippin was influenced by crop loads

of the prior year in a study by Buszard and Schwabe (1995). According to the results of their research, defruited trees in the previous prior year had flowers that were receptive to pollen at opening time while trees that carried a heavy crop load in the previous year had flowers that were not fully receptive to pollen until 3 DAA. We hand-pollinated uniform and single flower inflorescences during the study, meanwhile, Gonzalez *et al*. (1995) did not mention about flower quality. In addition, Gonzalez *et al*. (1995) used the pollen of male C for hand pollination, however, in our study, 'Tomuri' pollen was applied for pollination. The number of seeds is in turn related to the number of viable pollen deposited on the stigma. Even though a successful mating of a pollen tube with an ovule produces a seed, the number of pollen grains needed per seed ranges from 3.0 to 5.2 depending on the male clones (Hopping and Martyn, 1990). This is because some pollen tubes die during the passage through the style.

In this study, no differences in fruit set, seed number and weight were observed between 1-4 DAA. However, differences were observed for fruit weight, size, and density. Fruit weight reduced about 10 and 20 g in 2 and 3-4 DAA respectively. However, fruit weight between 1-4 DAA was ≥85 g and had more than 1200 seed per fruit. Gonzalez *et al.* (1998) observed similar results in *A. deliciosa* 'Hayward'. They detected the bulk of the fruit produced by *A. deliciosa* 'Hayward' with hand pollination were 80-110 g and stated that hand pollination increased the final value of the crop by 10%. Despite no differences in the number of seeds, average fruit weight was reduced from 10 to 20 g between 2-4 DAA. Under the terms of the similar fruit set, fruit weight will not be the same. Similar results were reported in *Actinidia chinensis* 'AU Golden Sunshine' by Thompson (2014).

Gonzalez *et al.* (1998) reported while fruit set is similar to that obtained with hand pollination and mechanical system, a high difference was obtained in fruit quality, where pollination hand still significantly improved mechanical pollination in terms of fruit size and weight. On day 5, not only fruit weight, but also seed number and weight reduced 34 g, 720, and 0.96 g, respectively. Thus, about 60% reduction of seed number on day 5, resulting reduction of fruit weight and size about 31%. Because seed-derived hormones are required to promote fruit growth (Woolley *et al.,* 1988). However, the final fruit size is also affected by the management factors such as crop loadings, nutrients, irrigation, time of anthesis, flower quality, pollination systems, beehives management, type of training systems, the position of the fruit on the vines in any particular training system and leaf to fruit ratio (Sale, 1981; Clinch, 1984; Hopping, 1986; Pyke and Alspach, 1986; Woolley *et al*, 1988; Ferguson, 1990; Gonzalez *et al.,* 1998; Goodwin, 2000).

There are several published relationships between the fruit weight and the number of seed or seed dry weight per fruit (Hopping, 1986; Pyke and Alspach, 1986; Testolin *et al.,* 1991; Goodwin, 2000). According to these researchers, to obtain a size of 70 g and 100 g fruit, 180 to 840 and 620 to 1290 seeds per fruit are required respectively. The seed-to-fruit relationships from these sources display very large scatter and either linear or non-linear positive correlation. Seed number may not be the only factor that plays a role in increasing fruit size. This is thought due to the influence of other factors such as the loading of the vine (Testolin *et al.,* 1991), cultural methods and orchard microclimates (Clinch, 1984), the age of flowers, storage carbohydrates, leaf number, and unknown factors should also be considered. Based on the findings of this study, not only fruit set but also fruit traits of 'Hayward' cultivar were affected by pollination in relation to the time elapsed since anthesis. Therefore, pollination efforts for *A. deliciosa* 'Hayward' may be concentrated within the first 4 DAA for having high fruit set and fruit traits. However, the maximum fruit size and weight obtained within the first DAA in this research.

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