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An Unmanned Aerial Vehicle Based Artificial Pollination in a Frost-affected Walnut (*Juglans regia L.)* Orchard

Dilan AHİ KOŞAR[a], **Eküle SÖNMEZ**[a], **Adem ARGAÇ**[b], **Ümran ERTÜRK**[a]

[a]*Department of Horticulture, Faculty of Agriculture, Bursa Uludağ University, Bursa, Turkey*
[b]*Maycev Seed Industry and Trade Limited Company, Bursa, Turkey*

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Corresponding Author: Ümran ERTÜRK, E-mail: umrane@uludag.edu.tr
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ABSTRACT

The winter and spring frosts pose a significant problem in many walnut cultivation areas as frost damage to catkins and female flowers results in low fruit set and yield. In January 2021, the temperature dropped to -17.2 °C in Yenişehir, Bursa, an important walnut production area in North-Western Turkey. The present study was carried out to determine the natural frost damage on catkins of some walnut cultivars and the effectiveness of an unmanned aerial vehicle (UAV) pollination, which has been developed and used in artificial pollination studies recently, on fruit set and fruit characteristics. For this purpose, two pollen concentrations (T1: 5% pollen two times; T2: 5% pollen in the first, 20% pollen in the second time) and the open pollination (control) were tested. Observations showed that frost damage on catkins varied from 57.31% (Ronde de Montignac) to 99.33% (Franquette). The initial fruit set was significantly higher in the T1 (87.74%) followed by T2 (83.89%). The final fruit set in T2 (75.16%) was higher than the T1 (74.11%), but the difference was found to be insignificant. The box and whisker plot shows that UAV pollination treatments (T1, T2) increased the fruit set, although this not uniform on the tree compared to open pollination. The highest nut weight, thickness, and length were obtained from control, followed by T1. The results showed that the fruit set was higher in pollination with UAV, and using less pollen (T1) was sufficient for fruit set. The research results support the use of UAV treatment on supplementary pollination for walnut.

Keywords: Agriculture drones, Winter frost, UAV, Pollen, Supplemental pollination, Fruit set, Catkins

1. Introduction

Juglans regia L., a species of nut, has a high degree of plasticity and can withstand cold winter temperatures in various regions without suffering winter injuries. However, its cultivation in the Mediterranean region is more limited due to its sensitivity to intense winter cold (Gandev 2013). Some cultivars may not show much damage during the cold winter between -9 °C and -11.5 °C. However, at lower temperatures (mainly when temperatures fall below -20 ºC), they are damaged and begin to dry from the tips of the branches (Şen 2011). Low-temperature damage differs between walnut species and cultivars, as well as between different organs and tissues (Lapins 1961; Malone & Ashworth 1991; Takeda et al. 1993; Aslamarz et al. 2010). Previous studies have determined some data belonging to frost-damaged walnut tree organs with artificial tests (Rochette et al. 2004; Poirier et al. 2010; Charrier et al. 2013; Aleta et al. 2014). Charrier et al. (2013) reported that buds were resistant but remained more sensitive than other organs in winter. However, frost damage observed in catkin buds was only found in Gandev's (2013) research on natural frost events. Gandev (2013) noted that in some cultivar's the male buds were more susceptible to winter frost than female buds in Bulgaria.

During active growth, the frost tolerance of tissues is relatively low, but when growth stops, cold-hardiness develops from fall to winter (Aslamarz et al. 2010). Recent developments in climate simulations indicate that climate change negatively affects winter chilling hours, and dramatic declines in cold-hardiness are predicted over the following several decades (Luedeling et al. 2011; Charrier et al. 2013; Vahdati et al. 2019, Özcan et al. 2019), which affects plants' cold acclimation and frost tolerance (Shepherd 2016; Liu et al.

2019). Warm spells may cause early dehardening, which could increase the risk of further freezing injuries in the late winter and early spring (Pagter & William 2011 and lead to damage in walnut shoots and buds, particularly male catkins. The lack of pollenizer resulting from damage to catkins causes a decrease in the natural pollination rate and fruit set. For this reason, supplemental pollination measures should be taken during the walnut blooming period. For that purpose, producers whose catkins have been damaged by winter frost collect healthy catkins from other orchards during the pollination period, putting them inside the net and hanging them on the branches to ensure wind pollination. Even though it is simple to utilize in small orchards, it wastes time and labor in commercial and industrial orchards. With this in mind, there is a need to consider artificial pollination as a supplemental pollination measure to increase the fruit set of walnut. Equipment used in pollination is significant since artificial pollination effectiveness depends on it. (Gianni & Michelotti 2018). There are two main approaches applied for artificial pollination equipment: aerial and ground platforms (Mazinani et al. 2021). A ground platform pollination system with electrostatic sprayers has previously been used to pollinate kiwifruit (Barnett et al. 2017; Williams et al. 2019), cherry (Whiting 2015), and date palm trees (Mostan 2012; Soliman et al. 2017).

Another approach, aerial platforms, including agriculture drones, have attracted attention because of their small size, convenient handling, and the way they reduce working hours (Lan et al. 2017; Kim et al. 2019). Agricultural robots include various unmanned aerial vehicle (UAVs) designs that spray pollen onto the crop canopies above. Pollination by wind power generated from the UAVs has been tested on rice (Wang et al. 2013; Li et al. 2015; Li et al. 2017) and sugar cane (Zhang et al. 2021). In addition, simulation flights were performed on walnut (Mazinani et al. 2021), and a pollen dump drone pollinating fruits (apples, cherries, and almonds) was recently developed by a New York-based startup (Matt Koball 2019). When considering UAVs pollination research, Matt Koball (2019) stated that controlling UAV flight height due to the uneven height of almond trees creates more challenges. Li et al. (2017) reported that the wind force generated by UAVs had been observed to disperse the rice pollen asymmetrically. In addition, simulation flights with UAVs in walnuts have shown that pollen spread may not be uniform in some cases due to wind effect. (Mazinani et al. 2021). However, no study has been found on the effect of pollination by UAVs and the amount of pollen used in pollination treatment on walnut fruit set and fruit characteristics. For these reasons, the present study seeks to determine the effectiveness of pollination by UAV as a supplemental pollination treatment on walnut orchards due to catkin damage in cultivars after a natural frost event.

The objectives were to 1) evaluate natural frost damage on male catkins of cultivars; 2) determine the effectiveness of UAV pollination on fruit set and fruit characteristics depending on the pollen dose used.

2. Materials and Methods

2.1. Observation of frost damage

The present study was carried out at Yenişehir, Bursa, (north-west Turkey; 40° 13′ 16″ N, 29° 31′ 04″ E latitude 40°11’ and longitude 29°3’) in 2021. The experiment plantation is characterized by 350 mm average annual rainfall and six months of frost danger (late November to April). The orchard was established in 2008 with a planting distance of 7x7 m and trained as a modified leader system. Catkins of seven (Chandler, Fernor, Ronde de Montignac, Cisco, Franquette, Meylanise, Kaman 1) cultivars grafted on *Juglans regia* were evaluated for natural frost damage. Temperature data were obtained from the automated climatic monitoring equipment during the frost period (Table 1).

Table 1- Minimum and maximum temperatures at the experimental orchard in the frost period

Frost period	*Minimum (°C)*	*Maximum (°C)*
January 17	-2.9	0.5
January 18	-16.0	-2.1
January 19	-17.2	-7.9
January 20	-16.7	-2.7
January 21	-10.8	1.8
January 22	-5.9	7.9

Seventy five catkins per tree were collected from 2.0-2.5 m above the ground, on four sides of the tree one month after the frost incident. The experiment was designed as randomized blocks with three replicates and two trees per replicate. Catkin buds were left for slow acclimatization at a low temperature (4 °C) for 24 hours, after which the temperature was gradually (16 hour) increased to 20 °C. To determine the frost damage percentage (FDP) of catkins, the buds were cut with a scalpel.

The observation was scored for visible symptoms of frost damage on a 1-4 scale: 1- completely green, living bud; 2- largely green bud; 3- partially green, browning bud; 4- dead bud (Figure 1). Following this, the frost damage index (FDI) was calculated according to the following formula: $FDI= \sum (n_i \times i)/N$, where "$n_i$" is the number of buds receiving the mark "I" (from 1 to 4) and "N" is the total number of buds in each cultivar. In addition, the FDP was recorded according to the presence or absence of buds damage.

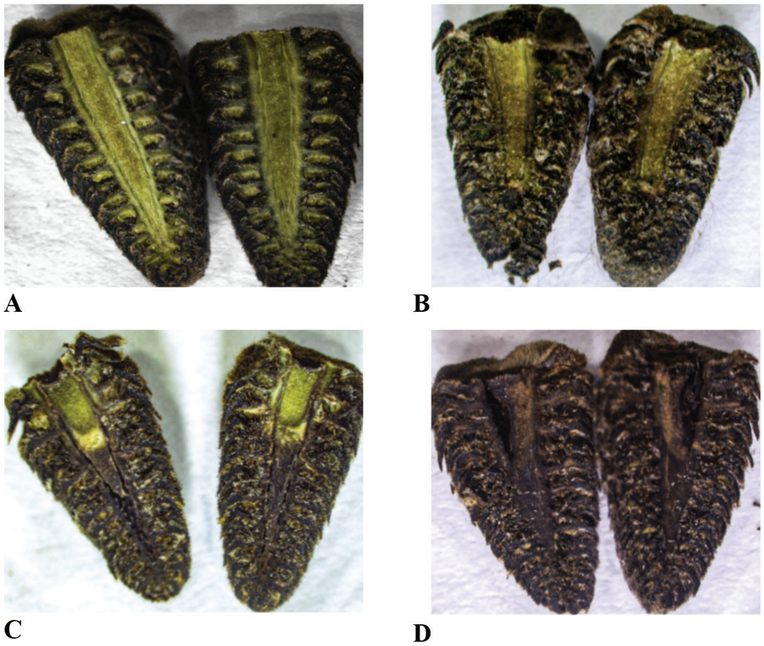

Figure 1. Visible symptoms of frost damage on young male catkins: (A) 1- scale, completely green bud; (B) 2- scale, largely green bud; (C) 3- scale, partially green; (D) 4- scale, dead bud

2.2. Artificial pollination with UAV

Chandler trees, the main cultivar in the orchard, were used in artificial pollination experiments. In the artificial pollination experiments, Chandler catkins were collected from the Agricultural Application and Research Center of Bursa Uludağ University's walnut orchard. Catkins were collected plump and yellow before they shed their pollen at the phenologic period termed the Fm stage (UPOV 1994). They were kept in a sieve in a warehouse at room temperature to gain pollen. Every day, the sieves were checked, and some calcium chloride was placed inside the containers to absorb moisture. The pollen that passed through the sieve was gradually kept at -20 °C until the female flowers were in the receptive state. The pollen was diluted with flour (w/w) (Kuru 1995) to obtain 5% and 20% (weight/weight) concentration (Figure 2). To estimate the viability of applied pollen, the 1% 2,3,5-triphenyl tetrazolium chloride method was used (Mert 2009; Özcan et al. 2017). The viability of pollen was determined by counting approximately 150 pollen using four replicates under the light microscope (×40; Leica DC 500, Wetzlar, Germany). A red color was considered viable, light red was semi-viable, and white pollen was non-viable (Mert 2009).

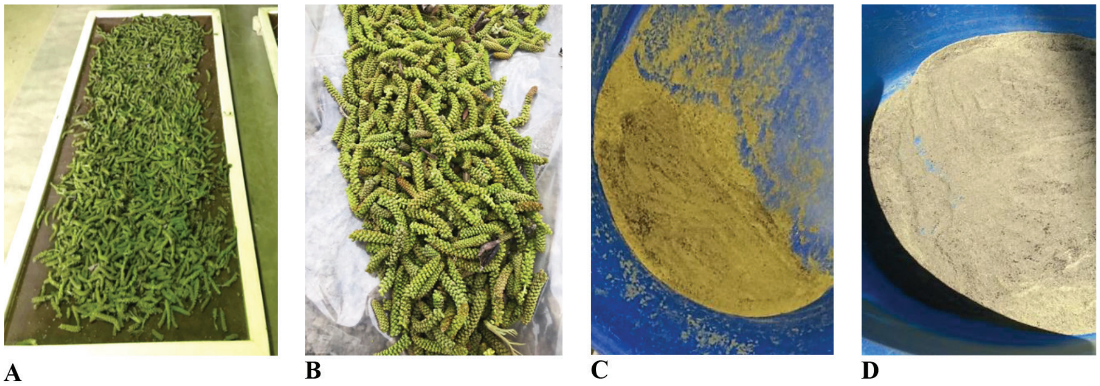

Figure 2. The pollen procedure: (A), (B) catkins in the sieve; (C) pollen; (D) pollen and flour mixture

Artificial pollination was repeated twice due to the gradual stigma receptivity. The treatments were composed of 5% in the first and second pollination (T1), 5% in the first, 20% pollen in the second pollination (T2), and open pollination (control). Trees belonging to the treatments were selected in different areas (Figure 3). The distance of the control area to the T1 and T2 treatment areas was 503 m and 731 m, respectively, and the T1 to T2 was 420 m. Trees for each treatment were selected according to the randomized blocks trial design with three replications and two trees per replication before the pollination. Three branches were marked from three sides and the middle layers of each tree. The 25 female flowers labeled per branch and 150 female flowers per replication were counted during female receptivity. The female flower and fruit marked branches were counted 25, 55, and 95 days (at harvest) after the pollination treatments, and the final fruit set rates were determined.

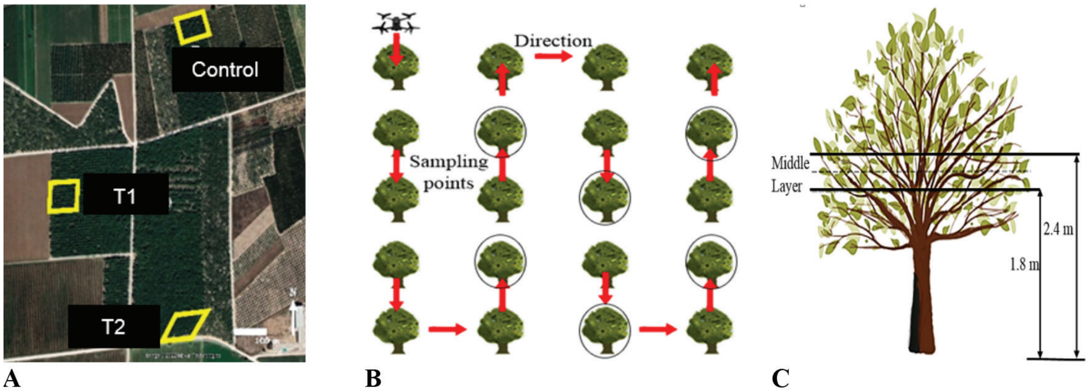

Figure 3. Artificial pollination procedure: (A) Experiment areas; (B) flight information for the walnut tree; (C) tree's middle layer where female flowers are marked. Note: This image is only for illustration

The quadrotor UAV (DJI Matrice 200 produced by Dà-Jiāng Innovations Science and Technology Co. Ltd Shenzhen, China) was used in the experiments. The quadrotor UAV has a 20 MP camera; it works with GPS navigation systems. The maximum ascent speed can reach up to 5 m/s and has a simple structure, autonomous obstacle avoidance, and program control. The specific technical parameters are shown in Table 2.

Table 2- Technical parameters of DJI Matrice 200 quadrotor UAV

Item	*Parameter*
Size (mm)	716×220×236 mm
Number of rotors (number)	4
Motor model	DJI 3515
Battery (mAh)	7660 mAh
Battery (number)	2
Maximum flying time (min)	13
Maximum ascent and descent speed (m/s)	5;3
Maximum wind resistance (m/s)	12
Maximum load (kg)	1.61
Operating temperature (°C)	-20 °C to 45 °C

The UAV, equipped with a 1-liter distribution kit, was produced by BLY-A matica and can pollinate 20 acres in a single flight (Figure 4). DJI APP software was used to calculate general parameters such as altitude, speed, battery information, and distance to the take-off site. All data specified with this software range 20 km, and an Android-based tablet was used as a mobile station.

The characteristics of the walnut trees, the weather conditions, and the UAV operation parameters during the artificial pollination experiment are shown in Table 3. The UAV was operated at 2 m above the canopy with an optimum flight speed of about 4 m/s (Meng et al. 2019).

Table 3- The walnut characteristics, weather conditions, and UAV operation parameters

Experiment time	*Phenological period*	*Tree height (m)*	*Wind speed (m/s)*	*Mean temperature (°C)*	*Drone flight height (m)*	*Drone flight speed (m/s)*
May 03 May 06	Stigma receptivity	12±0.60	0.78±0.40	18±1.50	3	4

The GPS coordinates were entered autonomously in the mobile station, the experiment orchard's planting frequency (7x7 m) was recorded, and the UAV dispersed the pollen on the rows via the kit. After beginning from the start of the row and finishing that row, the drone spread pollen from the end of the other row to the beginning, as shown in Figures 3 and 4.

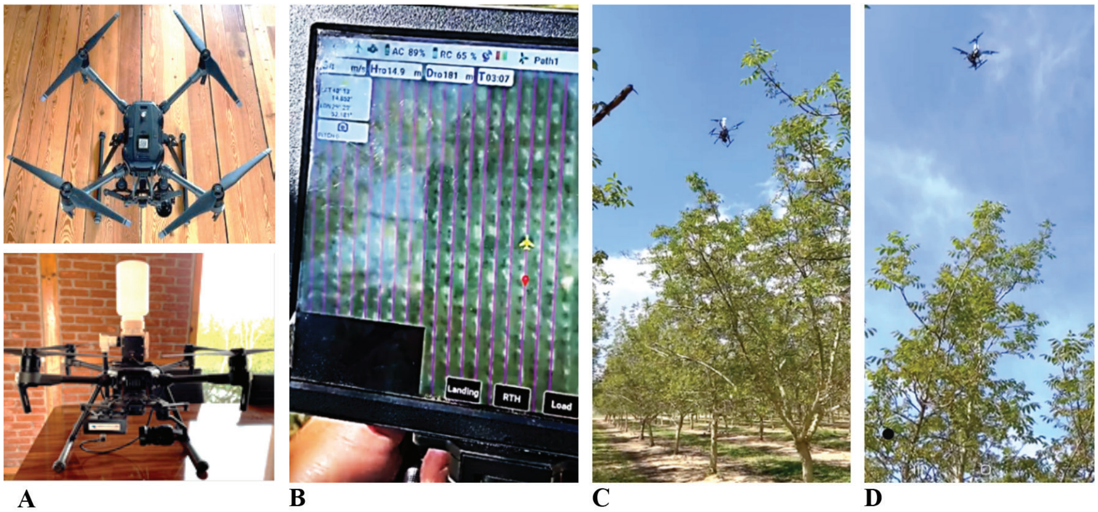

Figure 4- Pollination by UAV: (A) DJI Matrice 200 quadrotor UAV; (B) GPS coordinates entering on the tablet used as a mobile station; (C), (D) Polen disperse via kit on rows

2.3. Pistil squash method

Ten days after pollination, 8 female flowers were collected per replicate and fixed in FAA solution. Pistils fixed in FAA were washed three times with distilled water. The stigmas and the styles were separated from the ovary for examination. They were softened in 7% sodium sulfite by boiled for 50 min and then washed in distilled water three times. Afterwards, they were stained with lacmoide 0.2%, and kept for one night. The stained stigma and styles were placed on the slide and covered with a coverslip by dripping one drop of glycerin and lightly squashed. Pollen tube formations were observed under a light microscope (Ayfer 1967).

2.4. Fruit traits

After the harvest, the walnut fruits were dried and stored at 25 °C until the analysis. Fruit traits were analyzed based on the nut's weight, length, thickness, width, kernel weight, shell thickness, and kernel percentage in 3 replications of 30 fruits per replication.

2.5. Statistical analysis

An analysis of variance (ANOVA) was used for statistical analysis performed by Minitab version 16.1. The Tukey test was used to make pairwise comparisons between pollination treatments. The significance level used was $p \leq 0.05$. The boxplots were comprised using Minitab version 16.1 to identify the dispersion of the data set.

3. Results and Discussion

3.1. Observation of frost damage

A severe midwinter frost caused damage to the walnut cultivars' male catkins (Table 4). The percentage of frost damage ranged from 57.31% (Ronde de Montignac) to 99.33% (Franquette). All cultivars with the exception of Ronde de Montignac and Kaman 1 showed a high rate of catkin damage. The catkin of Kaman 1 and Ronde de Montignac cultivar were generally greener with 1.94 and 2.25 FDI values, respectively.

Table 4- Evaluation of frost damage to catkins of walnut selection and cultivars

Cultivars	*FDP (%)*	*FDI*
Ronde de Montignac	57.31±4.64 b	2,25 b
Chandler	99.00±0.57 a	3,94 a
Cisco	98.33±1.52 a	3,96 a
Fernor	98.92±1.86 a	3,80 a
Franquette	99.33±0.60 a	3,96 a
Meylanise	96.00±2.65 a	3,78 a
Kaman 1	57.45±7.80 b	1,94 b

Each data value is represented as means ± standard deviation. Means within columns followed by different letters differ significantly ($p \leq 0.05$).
FDP: Frost damage percent, FDI: Frost damage index

Winter frost damage was observed in walnut cultivars catkins in varying degrees. The damage percent of cultivars with the exception of Ronde de Montignac and Kaman were found to be similar. Gandev (2013) observed 40% to 98% damage on catkins of walnut cultivars at temperatures ranging from -13 °C to -24 °C. Similarly, Aslamarz et al. (2010) reported that a midwinter frost (-17 °C) at the experimental orchard caused moderate tissue browning in the buds and stems of cultivars and genotypes. Aleta et al. (2014) and Poirier et al. (2005) reported that 50% of the bud sticks and stem sections of Chandler, Fernor, and Franquette cultivars were damaged at -24.5 °C, -21.3 °C, -20.9 °C, and -22 °C, -24 °C, -26 °C respectively, in their artificial frost test. In the present study, we observed that catkins were damaged at -18 °C depending on the cultivars. This case may be explained when considering, the longevity of the frost, the rapidity of frost, the stage of dormancy of the trees, and that frost resistance varies by organs (Webster & Looney 1996). Furthermore, the results may differ depending on whether the frost tests are performed in the field or in the laboratory (Lapins 1961; Malone & Ashworth 1991; Takeda et al. 1993).

3.2. Artificial Pollination with UAV

The results showed that pollination treatments affected the flower abscission and fruit set (Table 5). It was determined that 30.37% of flower abscission occurred 25 days after pollination in control, followed by T2 (15.15%). 55 days after pollination, the fruit abscission ratio ranged between 8.75% (control) and 12.76% (T1), but there were no significant differences between pollination treatments. According to the flower abscission result of control treatment, it is assumed that a lack of pollen caused abscission due to the low number of viable catkins that provide pollination close to the orchard. For regular fruit set, 10-18 pollen grains should penetrate the stigmas. (Kaveckaja & Tokar 1963). Following the control treatment, the highest flower abscission was obtained from T2 (Figure 5). The pistillate flower abscission (PFA) at T2 can be explained by a high dose of pollen on the stigma. Previous studies recorded that the pollen load and level of abortion of pistillate flowers have a positive relationship (Por & Por 1990; Polito et al. 1996).

Table 5- Flower, fruit abscission and initial, final fruit set of pollination treatments (%)

Treatment	*Flower abscission*	*Fruit abscission*	*Initial fruit set*	*Final fruit set*
Control	30.37±0.59 a	8.75±4.56 ns	69.05±0.59 b	59.82±2.92 b
T1	11.64±3.29 b	12.76±5.95 ns	87.74±4.29 a	74.60±5.97 a
T2	15.15±8.00 ab	9.06±5.78 ns	83.89±8.00 ab	75.16±5.72 a

Each data value is represented as means ± standard deviation. Means within columns followed by different letters differ significantly ($p \leq 0.05$)

McGranahan et al. (1994) cited that lower flower abscission has been reported with few pollen grains on the stigma, which may be the result of less competition between pollen tubes in the pollen tube pathway. Similarly, Gonzalez et al. (2018) found that 1% pollen application with 5 pollen grains per mm^2 on the stigma surface provided maximum fruit set, and flower abscission increased with the increasing pollen concentration. Gün et al. (2010) reported that the PFA was 48.6%, 54%, and 77% when they controlled the pollination of female flowers with 5%, 50%, and 100% pollen.

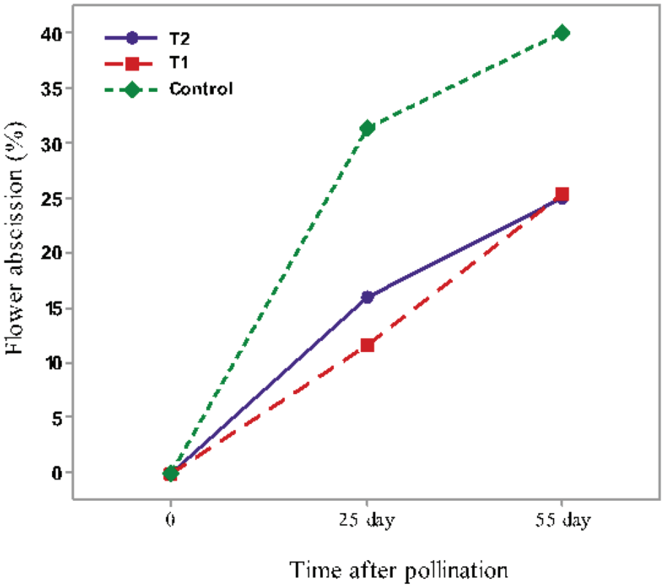

Figure 5- Flower and fruit abscission 25 and 55 days after pollination

41% of the pollen from the Chandler cultivar used in UAV pollination was found viable and 40% semi-viable. According to the pistil squash method, in open pollination (control) treatment, germination was not uniform across the stigmatic surface. In some areas, germination frequency was high, while another part of the stigma lobes had little germination. In the stigma lobes of the T2 treatment, numerous pollens were observed, and many of them were germinated. Pollen germination frequency was high in styles belonging to T1 and T2 treatment, and it was observed that some pollen tubes were very close to the ovary. The squashed pistil verified that the UAV airflow field could help the pollen spread and pollen germination (Figure 6).

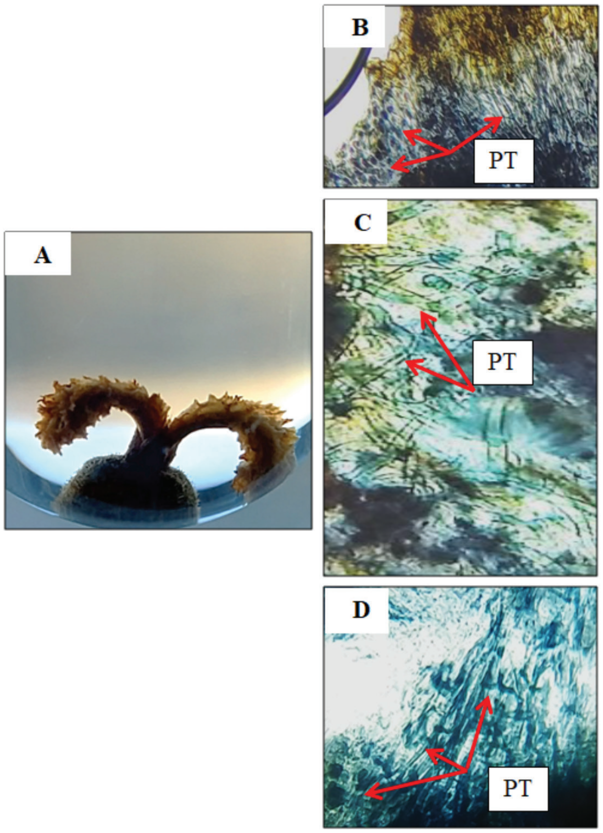

Figure 6- Pollen germination and pollen tube growth: (A) T1; (B) T2; (C) open pollination; (D) pollen tube formation (4x10)

The initial fruit set was found to be significantly high in the T1 (87.74%) and T2 (83.89%). The mean values of T1 and T2 were 21.30% and 17.68% higher than control, respectively. Similarly, the final fruit set was significantly higher in T2 (75.16%) and T1 (74.60%) compared with control (59.82%).

UAV pollination treatments increased the initial and final fruit sets compared to open pollination. The initial fruit set was greater than 80% in pollination with UAV, consistent with Lemus's (2010) findings that the best walnut yield is obtained when the fruit set reaches 80%. An initial and final fruit set in open pollination was found to be 69.05% and 59.82%, respectively. The distance of the control trees from pollination treatment areas (T1=503; T2=731 m) with the UAV suggests that the control trees did not receive pollen from these areas because the catkins of the Fernette and Franquette, which generally pollinate the Chandler cultivar, were damaged. However, the less damaged catkins of the Ronde de Montinag trees, about 420 meters from the control area, were able toollinate the last receptive female flowers of the Chandler cultivar. It has been reported that the pollen of walnut species can maintain its vitality when traveling distances of 1 km (Robichaud 2007), but the effective pollenizer distances rarely exceed 457 meters for Persian walnut orchards in California (Polito et al. 2005).

The pollination results with UAV are compatible with Mazinani et al. (2021), who reported the UAV could be used in walnut pollination successfully as a result of the simulation test. Similarly, Whiting (2015) studied artificial pollination in cherry trees using ground robots and revealed that it increased pollen density on flower stigma three-fold compared to pollination through bees. Liu et al. (2017) stated that UAVs could be used in supplemental pollination for hybrid rice seed production. In contrast, Wang et al. (2020) reported that UAV with low volume pollen spraying has varying particle size and coverage of the droplets deposited in the canopy of pear trees and that the fruit set rates were low. Also, Al-Wusaibai et al. (2012) reported that a higher date palm fruit set was obtained through manual treatment than mechanical pollination.

The box and whisker plot shows that 75% of the initial fruit set data of control treatment was less than 72.29%, and the values were distributed in a short range compared to T1 and T2. UAV pollination treatments increased the initial and final fruit set, but its variability was greater than open pollination (Figure 7). This may suggest that pollen might not be distributed evenly in the tree canopy during drone pollination. Liu et al. (2017) reported that the wind field produced by the rotor-wing UAV had an asymmetrical impact on pollen dispersal. Similarly, Mazinani et al. (2021) reported that pollen could not steer to walnut trees if the wind flow changes the path off the pollen. In quadrotors, the wind greatly influences their motion, making them unstable. The wind parallels to the flight direction are more useful for supplementary pollination (Mazinani et al. 2021; Zhang et al. 2021).

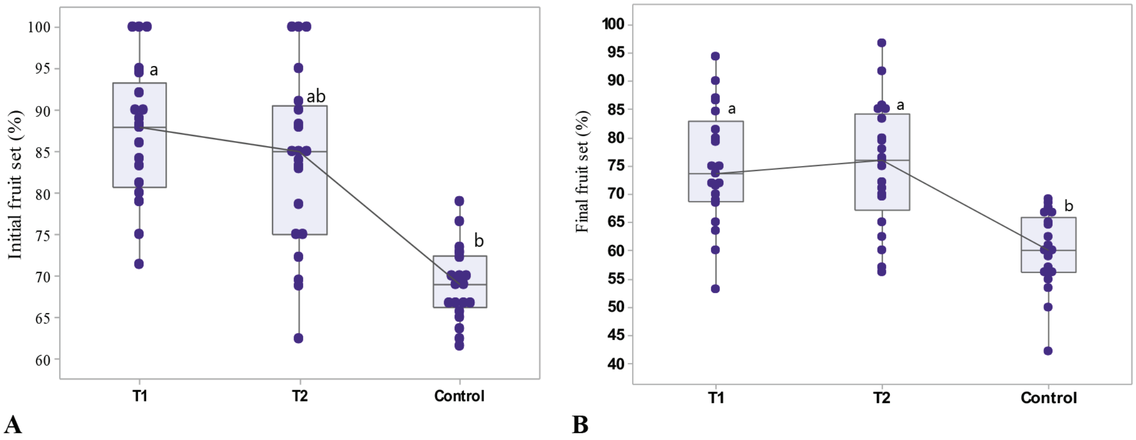

Figure 7- Boxplot graphs: (A) representing initial fruit set; (B) final fruit set value and its distribution in treatment. The central line displays the median, the bottom and the top of the box are the first and third quartiles, and the dots represent sample values. Lowercase letters are used to show statistical results of multiple comparisons treatments

Many previous studies reported that flight speed and flight height significantly impacted the pollen dispersal characteristics. Matt Koball (2019) noted that controlling UAV flight height due to the uneven height of almond trees creates more challenges. In the present study, it was tried to choose equal heights of trees to be pollinated by drone, the drone flight speed was set to be 4.0 m/s and the flight height to be 2 m above the canopy, as suggested in previous studies (Meng et al. 2019). In addition, attention was paid to selecting branches from the middle part of the tree. However, one of the reasons for the variation in fruit set could be that pollen distribution is not uniform since the height of the trees is not exactly uniform.

Furthermore, it has been reported in UAV spraying research that the tree canopy and the lower, middle, or upper parts of the tree affect the operation of agricultural UAVs. Zhang et al. (2016) reported that UAVs performed better when working on open center-shaped plants than round-shaped trees at a 1.0 m working height compared to other heights. Meng et al. (2019) also stated that with UAV spraying research comparing Y-shaped and Central leader peach, the droplet coverage in the upper layer was significantly higher than in the lower layers and the distribution was not uniform in the CL-shaped peach tree. Tang et al. (2018) reported that the operational height of 1.2 m and citrus trees with inverted triangle shapes yield optimal droplet deposition performance based on UAV spraying. According to the above mentioned research, in the present study, the high variability between the fruit sets obtained as a result of pollination with UAV may be related to the fact that the pollen on the layer from which the branches were selected may not be dispersed uniformly in relation to the flight speed, height and wind direction due to the height of the walnut trees.

The results showed that the highest initial fruit set was obtained from T1. Similarly, the highest final fruit set was obtained from T2, although there was no significant difference between treatments. Jameela & Alagirisamy (2021) reported that the date palm fruit set was significantly higher in liquid mechanical pollination, and there was no significant difference between the concentrations. Similarly, Akhavan et al. (2021) found no difference between pollen doses in fruit set in the date palm cultivar applied to different pollen doses through UAV material. According to the result of UAV pollination using 50 gr pollen in a 1-liter kit, 2 ha (120 trees) area was pollinated at once, and increasing the pollen dose did not affect the fruit set. Thus, it has been concluded that 5% of pollen treatment is sufficient for UAV pollination of the walnut trees. In the present study, approximately 0.5 mg of pollen was obtained from 1 catkin, and obtaining the pollen (50 g/L) required to pollinate 2 ha area simultaneously requires the collection of 50,000 catkins. Since it is known that a walnut tree can produce approximately 5000 catkins (Şen 1986), catkins must be collected from approximately 20 trees to pollinate 2 hectares of area with the drone. Considering that it is easy to harvest the catkins by shaking them mechanically and subsequently obtaining pollen, it would be reasonable to use this pollen dose for pollination.

The results showed that nut traits were affected by the pollination treatment (Table 6). The highest nut weight, thickness, and length were obtained from the control treatment. The kernel weight and shell thickness were unaffected by the pollination treatments. The kernel percent of fruits gave similar results in all treatments. Moreover, the nut length/width ratio (fruit shape) ranged from 1.20 to 1.24 and was unaffected by pollination treatment.

Table 6. Nut traits of the pollination treatments

Treatment	***Nut weight (g)***	***Nut width (mm)***	***Nut thickness (mm)***	***Nut length (mm)***	***Kernel weight (mm)***	***Kernel percent (%)***	***Shell thickness (mm)***	***Nut length/ width***
Control	12.47±0.26 a	33.30±0.32 ns	34.25±0.56 a	40.18±0.46 a	5.99±0.24 ns	47.26±0.92 ns	1.86±1.84 ns	1.20±0.20 ns
T1	11.53±0.43 ab	32.23±0.67	32.73±0.20 b	38.77±0.41 b	5.73±0.39	49.25±2.26	1.85±1.76	1.20±0.20
T2	11.29±0.53 b	32.24±0.41	32.55±0.29 b	40.06±0.12 a	5.31±0.38	46.40±1.94	1.92±1.91	1.24±0.20

ns: Not significant. Each data value is represented as means ± standard deviations of three replications. Means within columns followed by different letters differ significantly ($p \leq 0.05$)

The results of nut traits of the walnut cultivars are shown in Table 6. The nut weight, nut thickness, and nut length values were significantly higher in the control than in other treatments. Although the fruit setting of the drone pollination treatment (T1, T2) was about 20 % higher than the control, the control treatment fruits were heavier than others. Akhavan et al. (2021) observed almost similar findings in date palm pollination; the treatments with higher fruit set ratios had a lower fruit weight. Likewise, Awad (2010) reported that fruit diameter and length were significantly higher in traditional pollination than in spray pollination treatments. El-Mardi et al. (2007) reported no significant difference between hand pollination and mechanical pollination methods in fruit diameter in date palm cultivars, but fruit weight and length values were lower in mechanical pollination. In contrast, Razeto et al. (2005) reported that the fruit shape of kiwifruit was affected by the pollination treatment and the fruit size increased with machine pollination.

4. Conclusions

The present study determined that winter temperatures of around -17.2 °C damaged the catkins of walnut cultivars at different rates. Due to the effects of the frost, with the catkins being damaged, there was a lack of pollenizers in the orchard. The pollination of walnuts was tested using the UAV, which was attempting to be adapted to agriculture in the recent years, in order to find a solution to the problem which is experienced in the present study and may be experienced in the future due to climate changes. The findings of this study reveal that using UAVs on walnut trees could increase fruit sets by about 20%. Furthermore, increasing the pollen doses used in UAVs pollination did not increase the fruit set. Thus, a single operator using this machine could pollinate with 5% pollen doses more than 120 walnut trees per hour. UAV pollination did not affect the fruit quality but did affect fruit weight, thickness, and length. The fruits of open-pollinated trees were found to be heavier and larger than the others. These results are significant in showing the effect of UAVs pollination on walnut fruit sets and fruit characteristics. UAVs may require a significant initial investment, but fruit sets can be increased with the UAV pollination when catkins are damaged in commercial walnut orchards. Although pollination with UAVs increased fruit set, it was not uniform on trees compared to open pollination. For this purpose, to improve pollination efficiency, further experiments will be needed to determine the effects of the operation height and speed on pollen flow, including both the distance and intensity of pollen dispersal on different layers of walnut trees using UAV. In addition, considering that different pollen doses in the present study did not cause a difference in fruit set, pollination tests can be performed on walnuts by using less pollen in future UAV studies.

Data availability: Data are available on request due to privacy or other restrictions.

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