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# Pollination Biology of Cherry Laurel and Pollenizer Effects on Fruit Set and Fruit Characteristics

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**Abstract:** We investigated the fertilization biology of naturally grown cherry laurels and showed the effect of pollinizers on fruit quality. This is the first study on the pollination biology of cherry laurel (*Prunus laurocerasus* L.), conducted among three mothers and two pollenizer types employing *in vivo* open, self, and cross-pollination experiments. There was no difference in the pollen viability of different pollenizer types. The initial fruit set was monitored by counting fruits after 20, 40 and 60 days of the pollination (after flowers-small fruit drop, small fruit drop and June drop). The final fruit set was recorded in the second week of July at the beginning of the fruit ripening phenophase. The percentage of fruit set was calculated by comparing the number of developed fruits with pollinated flowers. In this study, the initial fruit set was very high but dropped severely in the next 40 days. The variations in the final fruit set were attributed to the pollen source effects. According to the results, genotype 16 was the best pollenizer for all mother genotypes. The fruit set increased with cross-pollination indicating that cherry laurel is self-incompatible. The pollen source significantly affected the fruit quality parameters.

**Keywords:** *Prunus laurocerasus* L., Pollen viability, Pollination, Fruit set, Fruit characteristics

## Karayemişin Tozlanma Biyolojisi ve Tozlayıcıların Meyve Tutumu ve Kalitesine Etkileri

**Özet:** Bu çalışmada karayemişin (*P. laurocerasus* L.) döllenme biyolojisi incelenmiş ve tozlayıcıların meyve kalitesine etkileri ortaya konmuştur. Karayemişin tozlanması üzerine ilk çalışma niteliği taşıyan bu çalışmada, üç ana bitki ve iki tozlayıcı bitki yer almış, *in vivo* koşullarda serbest tozlanma, kendileme ve karşılıklı tozlanma yapılmıştır. Denemede yer alan tozlayıcıların çiçek tozu canlılıkları arasında fark bulunmamıştır. Başlangıçtaki meyve tutumu tozlanmadan itibaren 20., 40. ve 60. günlerde (çiçek-küçük meyve dökümünden sonra, küçük meyve dökümünden sonra ve haziran dökümünden sonra) dalda kalan meyveler sayılarak tespit edilmiştir. Son meyve tutumu Temmuz ayının ikinci haftasında, meyve olgunlaşma döneminin başlangıcında tespit edilmiştir. Tozlamada yer alan çiçek sayısı gelişen meyvelere oranlanarak yüzde meyve tutumu belirlenmiştir. Çalışmada başlangıçtaki meyve tutumu çok yüksek olmuş, ancak tozlanmadan sonraki ilk 40 gün içinde şiddetli dökümler görülmüştür. Sonuçtaki meyve tutumu serbest, kendileme veya karşılıklı tozlanma durumuna göre, tozlayıcı kaynağından önemli oranda etkilenmiştir. Sonuçlara göre, genotip 16 tüm ana çeşitler için en iyi tozlayıcı olmuştur. Meyve tutumunun karşılıklı tozlanmada artması karayemişin kendine uyuşmaz olduğunu göstermiştir. Polen kaynağı meyve kalite parametrelerini istatistiksel olarak etkilemiştir.

**Anahtar kelimeler:** *Prunus laurocerasus* L., Polen canlılığı, Tozlanma, Meyve tutumu, Meyve özellikleri

## Introduction

Cherry laurel (*Prunus laurocerasus* L.) is one of the evergreen fruit species native to South-Eastern Europe and South-Western Asia. The plant is distributed naturally on light, medium and heavy clay soils and shows a better pest resistance than many species (Frohne and Pfander 1984). Cherry laurel is a decorative plant when it is in full bloom; therefore, it is used in parks and gardens. It is also pH adaptable, grows well in full sun or deep shade, salt spray tolerant, and can withstand heavy pruning (Posta 2009). It

is a good diet fruit with a high satiety value, and a significant source of phenolic compounds and anthocyanins. Fresh leaves are used to prepare 'laurocerasus water' in pharmacies for antispasmodic and breathing diseases. It is getting popularity at a commercial scale in the United States, Europe and Turkey (Foley and Raulston 1994; Sulusoglu et al. 2011). The initial breeding studies started with the selection of superior cherry laurel genotypes from natural populations (Akbulut et al. 2007; Sulusoglu 2011; Macit and Demirsoy 2012). These studies have contributed towards the commercial use of this plant in artificial pollination. Pollination and fertilization play a major role in fruit set and fruit quality in most fruit species.

The interaction between male and female flowers contributes to the success of fertilization. The pollen performance significantly affects pollination; thus, pollenizer selection is the first step to get an adequate fruit yield (Mahanoğlu et al. 1995; Sütyemez 2011; Nikolic et al. 2012). Cherry laurel yield varies from year to year. The trees may have a high yield in one year followed by a low production in the following years. Field studies demonstrate that the most important factors that affect cherry laurel fruit set and drop are: late spring; frozen, rainy and spring weather during bloom; summer heat, drought and agro-technical management (Sulusoglu et al. 2011). Planting of commercial orchards would be possible with suitable combinations. Thus so far, there have been no studies conducted on cherry laurel pollination. In the present study, we investigated the fertilization biology and compatibility possibilities in cherry laurel, along with the effects of pollenizers on the fruit quality.

## Materials and Method

*Plant Material*

The study was conducted in the years of 2011 and 2012 and included three mothers and two pollenizer genotypes, grown in Kocaeli City, North-Western Turkey, and involved the selection of superior cherry laurel genotypes (Sulusoglu 2011). Selected trees were similar with respect to age, size, and fruiting status. The plants were irrigated throughout the year and were protected from adverse climatic factors. The mother trees selected for the study were: 'Cherry type' cherry laurel (Genotype 37) and 'Black type' cherry laurels (Genotype 24 and Genotype 25). The pollenizers used for the experiment were: Genotype 16 "black type' and Genotype 34 'cherry type'. The pollen collected from the mother types was used for self-pollination treatments in the experiments.

*Flower characteristics and flowering time investigations*

The flower characteristics such as cluster length, flower number per cluster, pistil, and stamen numbers per flower were determined for 40 flowers and clusters. Flowering dates were determined according to BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry identification keys of stone fruits (code 61, 65 and 69) (Meier et al. 1994) for two years (2011 and 2012).

*Pollen collection and viability tests*

Unopened flowers (white balloon stage) were collected in April from all sides of the trees and were immediately transferred to the laboratory. Anthers were isolated from flower buds and placed overnight on a black paper under incandescent lamp on a table. Pollen was placed in small glass bottles, lids were closed and wrapped with stretch film and were preserved in a refrigerator (4 °C) until use. Bottles were prepared separately for cross-pollination treatments of each genotype. Pollen viability of the genotypes was determined with TTC (2, 3, 5-triphenyl tetrazolium chloride) staining test.

*Pollination treatments, initial, and final fruit set investigations*

Artificial pollination was carried out to determine the ratio of fruit set. Previously opened flowers and non-emasculated small flowers were removed from each selected cluster. Flowers were emasculated at the white balloon stage and counted before the clusters were isolated (using parchment paper bags) to prevent possible bee visitation and entry of unwanted pollen. Cross- and self-pollination treatments were performed by opening the bags, and applying the appropriate pollen to the receptive stigmas of the flowers with a fine paintbrush. They were randomly allocated to the treatment of cross-pollination with Genotype 16 and Genotype 34 pollens; self-pollination was done with their own pollen. Open pollination was designed to be natural without emasculation and bagging at the same time as artificial pollination.

After self and cross pollinations, the cluster was isolated using a parchment paper bag that was removed after petal fall.

The pollen was placed on the stigma in the third week of April 2011 and second week of April 2012. Initial fruit set was monitored by counting fruits 20 days after the pollination (after flowers and small fruit drop), second and third records were taken at the end of 40 and 60 days (after small fruit and June drop) and the final fruit set was recorded 'at the beginning of fruit ripening phenophase' in the second week of July. Percentage of fruit set was calculated by comparing the number of the set fruits with pollinated flowers.

*Fruit Characteristics*

The fruit characteristics of the genotypes such as fruit weight and seed weight of the fruits were determined for 40 fruits. Soluble solid content was measured using a hand held refractometer. The titratable acidity was measured by neutralizing the fruit juice to pH 8.2 with 0.1 N NaOH, and total acidity was given as a percentage of malic acid. Texture measurement was made in two different places in the equatorial region of the fruits with a handle penetrometer, with cone tip, as N/mm. Moreover, dry matter was determined by drying the samples in an oven at 105 °C (24 h) to a constant weight.

*Statistical analysis*

The experiment was set in a completely randomized plot design. For each combination of pollination treatments, fifteen flower clusters (of equal size and same phenological period) were chosen from the different sides of the trees as a replication and three replications were used. A total of 45 flowering shoots were used in each application. The fruit set was determined by comparing the number of set fruits with the number of pollinated blossoms as a percentage mean value of 15 flower clusters for each replicate. The data were analyzed using the analysis of variance (ANOVA), and the differences among mean values were determined using Duncan's test at $p<0.05$. Data containing percentage values were transformed to arcsin square root values before statistical analysis.

## Results and Discussion

*Pollen viability*

The knowledge of pollen characteristics and pollination ability helps provide high quality and regular yield. Perfect cultivar composition of plantation is the main factor for successful fruit production while high pollen viability is one of the major indicators of good pollen resource for a cultivar (Suranyi 2006; Nikolic et al. 2012). In this study, all of the cherry laurel genotypes studied showed high pollen viability and were a presentable pollen source (Table 1). On an average, genotype 37 showed the highest pollen viability (93.30%), whereas genotype 24 showed the lowest (90.41%). The differences in pollen viability between the two genotypes were statistically significant and the results were in agreement with previous pollen viability rates of cherry laurel determined by Sulusoglu and Cavusoglu (2013). Pollen viability was slightly higher in the second experimental year than in the first year. This result corroborates a previous study on plum cones (Horvath et al., 2000).

Table 1. Pollen viability (%) of cherry laurel genotypes as determined by TTC

| | **Pollenizers** | | **Mother genotypes** | | | |
|---|---|---|---|---|---|---|
| | **Genotype 16** | **Genotype 34** | **Genotype 24** | **Genotype 25** | **Genotype 37** | **Average** |
| **2011** | 89.06 | 89.56 | 89.94 | 91.66 | 91.81 | 90.41 |
| **2012** | 92.22 | 92.90 | 90.88 | 92.25 | 94.79 | 92.61 |
| **Average** | 90.64 | 91.23 | 90.41 | 91.96 | 93.30 | |

*Flowering Characteristics*

Cherry laurel flower buds appeared in early spring and opened from mid-April to the first week of May in 2011; and between the first and third week of April in 2012. The full flowering phenophase occurred

within four to ten days after the onset of flowering and had an average duration of 12 to 17 days. Flowering occurred earlier in the year 2012 than in 2011. Genotype 37 flowered earliest while Genotype 34 had the shortest flowering period in both of the experimental years (Fig. 1). Flowering time changes in response to the climatic factors, which is one of the main criteria for a perfect pollination. The phenological calendar showed differences during both the study years; however, the records showed that flowering periods in used mothers and pollenizer genotypes provided adequate overlap to enable pollination. These results were in concurrence with the findings of Krška (1994), who showed that the onset of the flowering can differ by 25 to 40 days from year to year depending on the cultivar and climatic conditions during the year.

We observed that cherry laurel had white solitary flowers that occurred diagonally in a flower cluster; the length of racemes varied from 8.20 to 10.77 cm (Genotype 16 and Genotype 24, respectively). Genotype 24 had the maximum flower number on a cluster with an average of 35.86 flowers. The flowers were perfect, and each flower comprised of five white petals, five green sepals, average 18.87-21.23 stamens (respectively for Genotype 34 and Genotype 16) and a pistil in the center of the flower (Table 2). The differences in flowering characteristics were not statistically significant among different cherry laurel genotypes.

Table 2. Flowering characteristics of cherry laurel genotypes

| | | Flower cluster length (cm) | Flowers numbers | Stamen numbers | Pistil numbers |
|---|---|---|---|---|---|
| **Pollenizers** | 16 | 8.20 | 32.11 | 21.23 | 1.0 |
| | 34 | 9.89 | 35.11 | 18.87 | 1.0 |
| **Mother** | 24 | 10.77 | 35.86 | 19.30 | 1.0 |
| **Genotypes** | 25 | 9.81 | 34.67 | 19.30 | 1.0 |
| | 37 | 9.37 | 32.17 | 19.20 | 1.0 |

*Fruit set and fruit drops*

The changes in fruit set and fruit drop, after 20, 40 and 60 days of pollination and at the harvest time in the second week of July, were recorded four times. The results showed that fruit set was strongly affected by the pollen source (Figure 2). Initial fruit set (20 days after the pollination) was relatively high in all combinations of the pollinations as shown in Fig. 2. This was previously reported in plum cultivars where the initial fruit set was relatively high (Ogasanovic 1985; Nikolic et al. 2012). The high fruit set in this period, reflected by the amount of fruit harvested, depends on the false fertilization effect (Ogasanovic 1985). The fruit drop was severe within 40 days after pollination in all the treatments. Moreover, fruit drop in the self-pollinated fruits continued dramatically until harvest time. Self-pollination resulted in a very low fruit set in all genotypes; and Genotype 25 and Genotype 24 did not show fruit set at harvest in 2012. Open-pollination resulted in close fruit set percentages among genotypes in the first year of the study (2011), while Genotype 25 yielded fewer fruits in 2012. On the other hand, cross-pollination exerted positive effects during initial fruit set in all genotypes; Genotype 25 had the highest "initial fruit set" with both Genotype 16 and Genotype 34 pollenizers. A severe fruit drop was observed in the first 40 days after the pollination, which decreased over time, and became insignificant near the harvest time (Figure 2).

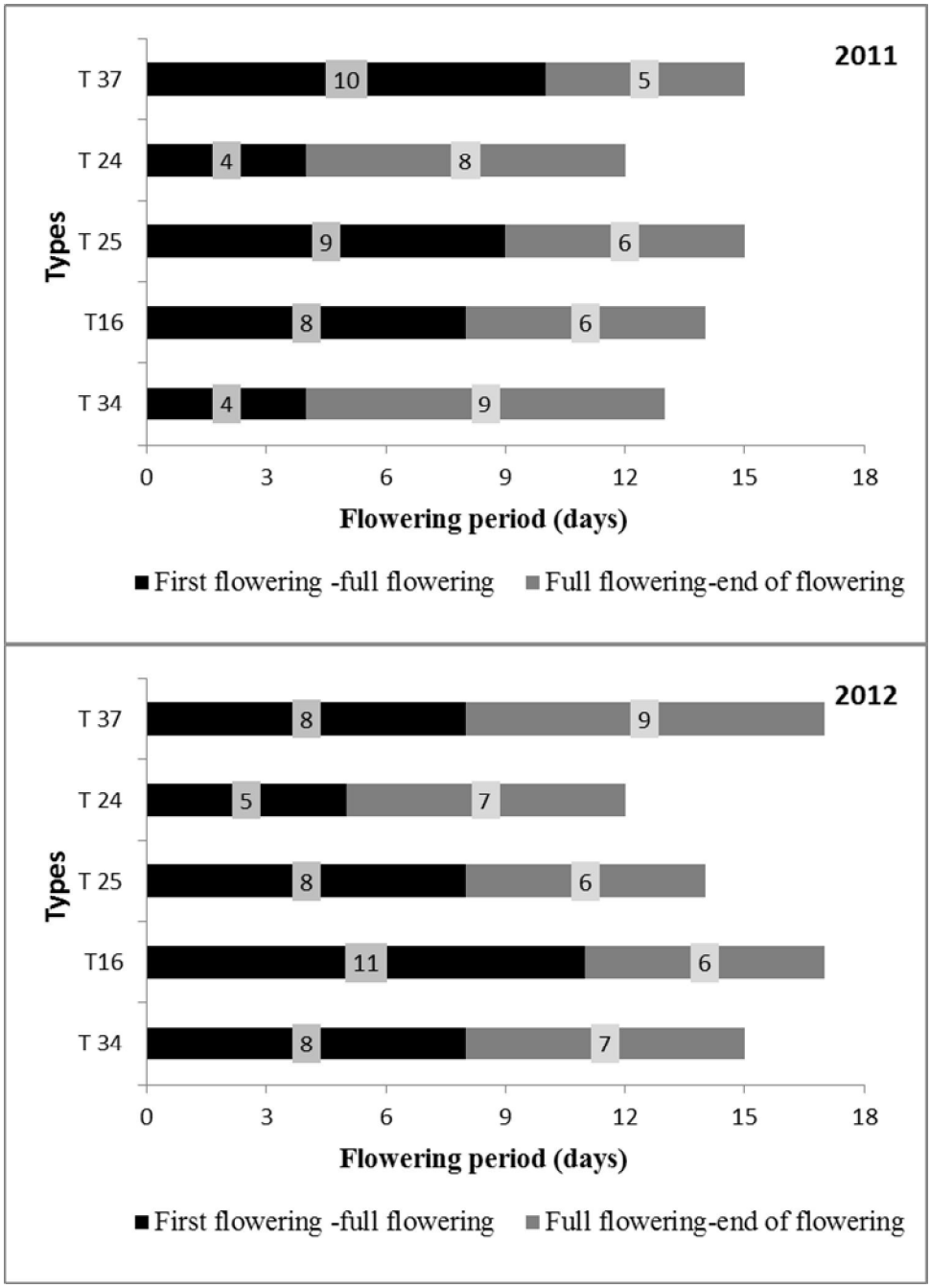


Figure 1. Flowering periods of cherry laurel genotypes

The percentages of the harvested fruits (depending on the crossing combination) varied from 1.05% to 69.24% in 2011; and from 0.00% to 77.06% in 2012 (Table 3 & 4). A significantly higher number of set fruits was obtained from artificial pollination of plum (Keulemans, 1994); which is similar to the number of harvested fruits in our study. The final fruit set was significantly affected by the pollenizers and mother genotypes in both the years. A higher fruit set with cross-pollination in Genotype 16 and Genotype 34 was obtained than self and open-pollination treatments in Genotypes 24 and 25. Similarly, Genotype 37 showed highest fruit set with Genotype 16 pollenizer, while Genotype 34 pollen gave fruit set similar to open pollination treatments. Fruit set was higher in 2012 than 2011 (34.28% and 27.55%, respectively), and Genotype 16 was found to be the best pollenizer for all the genotypes among cross-pollination (50.02% and 62.15% fruit set in 2011 and 2012, respectively). Fruit set varies with different pollenizers, and fruit set ratios of a species can change from 0.0% to 70% with different pollenizers (Arzani and Khalighi 1998; Sütyemez and Eti 1999; Sütyemez 2011). This has been frequently observed in many

other species (Kellerhals and Rusterholz 1994; Cuevas and Polito 1997; Botu et al. 2002; Koskela et al. 2010). Wertheim (1991) found pollination to be inconsistent across the years, which concurs with our study.

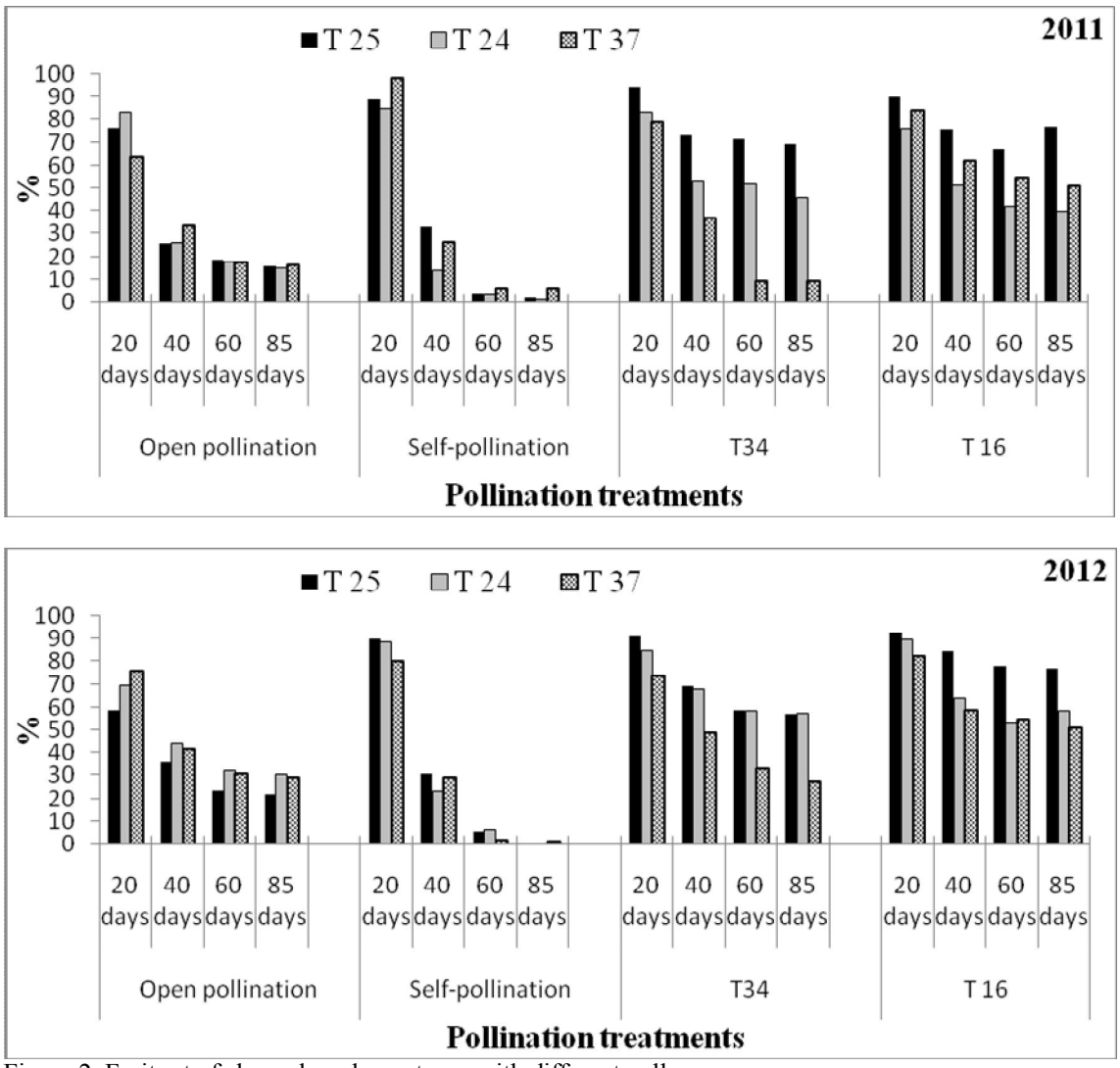


Figure 2. Fruit set of cherry laurel genotypes with different pollen source

However, Genotype 25 showed the highest fruit set (69.24%) in cross-pollination with Genotype 34 in 2011, wherein both the pollenizers exhibited similar effects on the fruit set. Genotype 34 pollen increased the fruit set of Genotype 25 in 2011, but this pollen source caused a significant decrease in the fruit set in 2012. The percentage of the fruit set in Genotype 37 was significantly higher when Genotype 16 was selected as the pollen source in both of the experimental years. Genotype 34 was not a good pollenizer for Genotype 37 and produced a low fruit set (Table 3-4).

Open pollination in Pandy sour cherries caused low fruit set that was highly variable during different seasons (Nyeki 1989). Moreover, there is a direct correlation between the rate of self-fertility and yield consequent to open pollination (Nyeki, 1989). In present study, a moderate amount of the fruit set was found with open pollination; and Genotype 37 also showed fruit set with self-pollination. Thus, the fruit set percentage varied on a yearly basis in all the genotypes. The maximum fruit set was only 37.2% and 44.2%, in self and open-pollination, respectively, of apricot cultivars (Pugliano and Forlani, 1987). The rate of fruit set in open-pollination (as affected by pollenizers) was lower than in cross-pollination (Mahanoglu et al. 1995; Nikolic et al. 2012).

About 25% to 30% of the flowers should set fruits for adequate yield (Davarynejada et al. 2014); however, in sweet cherry, 30% fruit set is described as "extremely high" (Bekefi, 2004). In our study, initial fruit set decreased with fruit drop but final fruit set was more than these ratios with both Genotype 16 and 34 pollenizers. It was observed that high fruit yield lowered fruit quality, which was indicated by small, poorly colored fruits that lost their round shapes.

Table 3. Final fruit set (%) of cherry laurel genotypes in 2011*

| Pollen source | Mother genotypes | | | |
|---|---|---|---|---|
| | **Genotype 24** | **Genotype 25** | **Genotype 37** | **Mean** |
| **Open-pollination** | 15.09 bA | 15.60 bA | 16.47 bA | 15.72 |
| **Self-pollination** | 1.05 cA | 1.92 cA | 5.77 cA | 2.91 |
| **Genotype 34** | 45.89 aB | 69.24 aA | 26.19 bC | 41.55 |
| **Genotype 16** | 39.62 aB | 62.22 aA | 48.23 aB | 50.02 |
| **Mean** | 25.41 | 37.25 | 20.00 | General Mean: 27.55 |

*Values in the same column (fruit set differences among different pollination treatments on the same mother genotype) with different lower-case letters are significantly different; values in the same row (fruit set differences among different mother genotypes at the same pollination treatment) with different upper-case letters are significantly different ($p<05$).

Table 4. Final fruit set (%) of cherry laurel genotypes in 2012*

| Pollen source | Mother genotypes | | | |
|---|---|---|---|---|
| | **Genotype 24** | **Genotype 25** | **Genotype 37** | **Mean** |
| **Open-pollination** | 30.50 bA | 22.08 cA | 28.93 bA | 27.17 |
| **Self-pollination** | 0.00 cA | 0.00 dA | 1.11 cA | 0.37 |
| **Genotype 34** | 57.48 aA | 57.34 bA | 27.51 bB | 47.44 |
| **Genotype 16** | 58.24 aB | 77.06 aA | 51.15 aB | 62.15 |
| **Mean** | 36.56 | 39.12 | 27.18 | General Mean: 34.28 |

*Values in the same column (fruit set differences among different pollination treatments at the same mother genotype) with different lower-case letters are significantly different; values in the same row (fruit set differences among different mother genotypes at the same pollination treatment) with different capital letters are significantly different ($P<05$).

*Fruit characteristics and effects of pollenizers*

The fruits were harvest and measured in July 2011 and 2012. The pollenizers affected the fresh fruit weight and seed weight. The open pollinated Genotype 24 and 25 fruits were heavier than cross-pollinated fruits, while Genotype 37 fruit weight increased with pollenizer Genotype 16 (Table 5). These results corroborate with previous studies (Mahanoğlu et al. 1995; Sütyemez and Eti 1999). Certain selected pollenizers caused changes in fruit characteristics such as soluble solid content, titratable acidity and fruit dry weight. Genotype 34 increased the acidity of fruits as compared with open pollinated fruits (Table 6). Cross-pollination changes fruit characteristics in many species (Sütyemez and Eti 1999; Karakas-Cırtlık and Beyhan 2012). The decrease in fruit firmness was not significant in fruits of Genotype 24 and 25 pollinated with Genotype 16 (black type cherry laurel) (Table 6).

Table 5. Effects of pollen source on fruit quality characteristics of cherry laurel genotypes

| Mother Genotypes | Pollen source | Fruit weight (g) | Seed weight (g) | Fruit Dry weight (%) |
|---|---|---|---|---|
| **Genotype 24** | Open pollination | 4.42 bA | 0.276 bB | 17.76 aB |
| | T 16 | 4.14 aB | 0.306 aB | 17.09 aA |
| | T 34 | 4.14 bA | 0.327 aA | 13.98 bB |
| **Genotype 25** | Open pollination | 4.63 aA | 0.351 aA | 13.41 bB |
| | T 16 | 3.66 bB | 0.314 bB | 16.98 aA |
| | T 34 | 3.78 bA | 0.297 bB | 15.02 bB |
| **Genotype 37** | Open pollination | 4.17 bA | 0.368 aA | 18.21 aA |
| | T 16 | 5.14 aA | 0.357 aA | 18.11 aA |
| | T 34 | 4.41 bA | 0.340 aA | 17.66 aA |

*Values in the same column (pollen source effects on mother genotypes) with different lower-case letters and values in the same row (mother genotype's effects on pollen source) with different upper-case letters are significantly different (P<05).

Table 6. Effects of pollen source on fruit chemical characteristics of cherry laurel genotypes

| Mother Genotypes | Pollen source | Fruit firmness (N/mm) | Soluble solid content (%) | Titratable acidity (malic acid %) |
|---|---|---|---|---|
| **Genotype 24** | Open pollination | 3.18 | 17.43 aA | 0.18 bC |
| | T 16 | 2.97 | 18.27 aB | 0.19 bB |
| | T 34 | 3.20 | 19.07 aA | 0.27 aB |
| **Genotype 25** | Open pollination | 3.69 | 16.53 aA | 0.23 bB |
| | T 16 | 3.41 | 17.77 aB | 0.31 aA |
| | T 34 | 3.74 | 17.17 aA | 0.32 aA |
| **Genotype 37** | Open pollination | 3.96 | 16.98 bA | 0.30 bA |
| | T 16 | 4.00 | 21.40 aA | 0.31 bA |
| | T 34 | 3.78 | 17.33 bA | 0.34 aA |

*Values in the same column (pollen source effects on mother genotypes) with different lower-case letters and values in the same row (mother genotype's effects on pollen source) with different upper-case letters are significantly different (P<05).

## Conclusion

This study investigated various cherry laurel genotypes that showed satisfactory *in vitro* pollen viability. The initial number of fruit set was very high and did not differ depending on the applied pollen source. On the other hand, there were significant differences among the genotypes and pollenizers during the final fruit set. This is the first study on the cross-pollination in the cherry laurel (*Prunus laurocerasus* L.) that established the requirement of a pollinizer in cherry laurel for a high fruit set. The effect of pollinizers upon the fruit characteristics was also manifested. Self-incompatibility in genotypes of cherry laurels was found, which has important implications for the plantation establishment of this fruit. This study will continue further focusing on utilization of new pollinizers, fruit set in different combinations of cross-pollination, and dynamics of pollen tube growth in pistil.

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