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Effect of pistil receptivity, pollen mixtures, and pollen application distances on fruit set of pistachios (*Pistacia vera*)

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Abstract This study was carried out at the pistachio (*Pistacia vera*) orchards of Ceylanpınar State Farm, Turkey. The cultivar 'Siirt' and Male 13 were used in the experiments. The aim of the study was to investigate the effect of pistil receptivity, pollen mixtures, and pollen application distances on fruit set using artificial pollination in pistachios. To determine the receptivity of female flowers, anthesis was accepted as day 0, and then one application was carried out on 0, 2, and 4 days after anthesis; and repeated applications were done on 0+2, 0+4, 2+4, and 0+2+4 days, separately. Pollen and wheat flour was mixed to obtain 1%, 5%, 10%, 50%, and 100% pollen mixture, and these pollen mixtures were sprayed from 25, 50, and 100 cm distances from the inflorescence clusters with a small hand sprayer. Thus, fruit set was determined for the applications. Different pollen mixtures were applied to a microscope slide, covered with Vaseline, from three different application distances to determine the number of pollen grains per unit area by artificial pollination. The number of pollen grains per unit area was counted under a light microscope and calculated per 1 mm^2. Finally, adequate pollen numbers to pollinate a flower were determined. The results indicate that for optimum fruit production of pistachio, 5% and 50% pollen mixtures should be sprayed at a distance of 100 cm at 0 and 4 days after anthesis. The adequate number of pollen grains to pollinate a flower was calculated as 14 or 15 pollen grains per flower in pistachio. Increased number of pollen per stigma area would cause excessive flower abscission after a certain point, and this could be the cause of low fruit set in pistachios.

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INTRODUCTION

The pistachio (*Pistacia vera* L.) is a dioecious and wind-pollinated fruit species. The pistillate and staminate flowers are produced in large inflorescences on different trees. Pollination is important for this species; the marketable part is the seed and to obtain good fruit set, adequate numbers of suitable male trees have to be interplanted in the orchards with consideration given to wind and rain conditions.

Pistillate flowers are small and their size is 2–3 mm. The style is short and the stigma is big, three-partitioned, uneven surfaced, and its surface is 3 mm^2. The ovary is elliptical, oval, or round shaped as is the fruit (Kaska et al. 1989).

Most of the pollen grains should exist in the environment 2 or 3 days after anthesis for adequate fruit setting (Crane & Maranto 1989; Shuraki & Sedgley 1994; Acar 2004).

The pollinator is a main factor in pistachio productivity. Adequate pollinators (male trees) have to be interplanted in the orchards to obtain good fruit set. Commercial pistachio orchards usually contain one male tree per 8–11 female trees. However, there are significant pollinator problems in pistachio orchards in the south-east Anatolian region of Turkey. Suitable pollinators were determined (Atli et al. 1995; Acar 1997, 2004) but they are not widespread. Pollinators have been found to be effective in fruit set and they could be a factor in flower and fruit abscission in pistachio (Acar & Eti 2007). Fruit drop can also be influenced by the timing of pollination, either in relation to flower age or environmental conditions at anthesis such as temperature (Crane & Iwakiri 1981). Pollination was found to be a limiting factor in immature pistachio trees. It is suggested that, in some instances, electrostatic pollination could supplement natural pollination, thereby ensuring higher yields with better nut quality. However, poor levels of pollen alone could not account for all the differences in cropping between immature and mature pistachio trees (Vaknin 2006). Electrostatic forces have been shown to influence deposition in agricultural wind-pollination settings when the pollen is artificially charged. When pollination is a limiting factor, electrostatic pollination of pistachio can increase yield and may even increase fruit quality. However, when pollination is not a limiting factor, electrostatic pollination may even reduce yield (Vaknin et al. 2001, 2002).

Pollen diluents would extend the use of collected pollen and decrease the amount required and the cost of collection. Corn starch, rice and wheat flour were convenient pollen diluents for artificially pollinating pistachio; however, wheat flour was used successfully. Talc was about the same size as pistachio pollen and it stuck on the surface of the atomiser tank (Kuru 1995). Pollen combined with wheat flour, polyester, or Rilsan ES powders had the same *in vitro* germination percentages and tube numbers in the style; differences in physical characteristics among the powders may impact on their application. Wheat flour and polyester powders had no detrimental effect on pollination (Weiguang et al. 2003). The time of pollination of the female flower after first opening has been shown to be a significant determinant of yield (Shuraki & Sedgley 1994).

To pistachio growers, male trees seem undesirable because of their lack of fruit. Pistachio growers are not aware of the importance of pollinators for fruit set and they also complain of inadequate fruit set. They have been informed lately about the importance of male trees. In this instance, the pollinator problems of orchards may be solved with the transformation of some female trees to male ones by grafting. These transformed trees can start pollination at least 3–4 years after grafting. Where there are inadequate suitable pollinators in the pistachio orchards and in newly transformed male trees, artificial pollination is the sole solution to fruit set. Application is easy because of the abundance of pollen in the flower clusters of male pistachio trees and its ease of extraction from the clusters. However, three questions arise: (1) how much pollen will be applied from which distances?; (2) which stage of anthesis will be more suitable for artificial pollination?; and (3) does more pollen mean more fruit set?

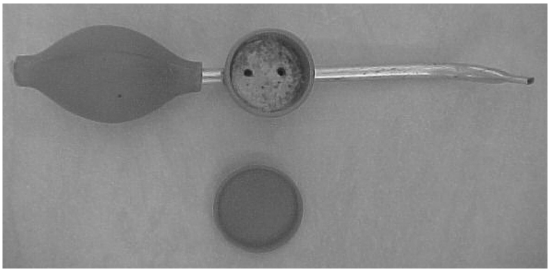

Fig. 1 Hand sprayers used in the pollination treatments.

The aim of this research was to investigate the effect of pistil receptivity, pollen mixtures, and pollen application distances on fruit set, and to determinate the effectiveness of artificial pollination in pistachios.

MATERIALS AND METHODS

Plant material and sampling

This research was carried out at Ceylanpınar State Farm in the Şanlıurfa province of Turkey. 'Siirt' pistachio and Male 13 were used in the experiments to determine the effects of pistil receptivity, pollen mixtures, and pollen application distances on fruit set using artificial pollination. Pollination was conducted in 2000 and in 2001.

Wheat flour was used as the diluent and pollen of Male 13 was mixed with wheat flour at a 1:99 (w/w) ratio for 1% pollen; 5:95 for 5% pollen; 10:90 for 10% pollen; 50:50 for 50% pollen; and 100:0 for 100% pollen mixtures before using artificial pollination to attain better dispersal.

Pollen mixtures were sprayed from three application distances (25, 50, and 100 cm) from the inflorescences with hand sprayers (Fig. 1), and another hand sprayer was used for each pollen mixture.

Inflorescences that had some flowers with dehiscent anthers were removed from the Male 13 tree, brought into the laboratory, and spread over tissue paper. Pollen that was shed overnight was sieved and collected in laboratory conditions. Pollen germination percentages *in vitro* with the hanging-drop technique (in 20% sucrose concentration) at anthesis were 72.26% and 75.16% for Male 13 in 2000 and 2001, respectively.

Pollen and wheat flour was combined to obtain pollen mixtures, and the mixtures were sprayed at three distances from the inflorescence clusters with small hand sprayers. Anthesis was accepted as 0 day, and one application was done on 0, 2, and 4 days after anthesis; and repeated applications were carried out on 0+2, 0+4, 2+4, and 0+2+4 days, separately. Pollinated flowers were re-bagged after hand pollination.

Five pollen mixtures, three pollen application distances, and seven pistil receptivity pollinations (0, 2, 4, 0+2, 0+4, 2+4, and 0+2+4 days after anthesis) were used in the study. For each application, eight inflorescences randomly marked from four different sides of trees to increase replication were selected and tagged on three trees; in this way, 840 inflorescences were used on 15 female trees in the research. Inflorescences were bagged before anthesis to exclude foreign pollen.

Fruit set

Fruit set was determined as the final fruit number/initial flower number as a percentage. Individual flowers on pistillate inflorescences were first counted 1 week after pollination. Then fruits on inflorescences were counted at harvest to determine fruit set for pistil receptivity, pollen mixtures, and pollen application distances. Average fruit set was also determined as 5.26% at the natural wind-pollinated trees, but could not be compared with the other applications because of the lack of pollen application distances and pistil receptivity in the natural pollination.

Pollen density

Different pollen mixtures (1%, 5%, 10%, 50%, and 100%) were applied to a microscope slide, covered with Vaseline, from three distances (25, 50, and 100 cm) to determine the number of pollen grains per unit area in artificial pollination. Pollen grains were counted under a light microscope using an ocular square micrometer and calculated per 1 mm^2. Finally, adequate pollen grain number to pollinate a pistachio flower was determined.

Statistical analysis

The data were analysed by ANOVA (analysis of variance) test from the statistical package MSTAT (Michigan State University, United States). Tukey test was used for mean separation. Significant differences were determined at $P \leq 0.05$.

RESULTS

Fruit set

Pollen mixtures and application distances affected fruit set (Table 1). The best fruit set was obtained from the fruit clusters when 5% pollen mixtures were applied at 50 and 100 cm, and 10% and 50% pollen applied at 100 cm. Pure pollen caused the lowest fruit set when applied at 25 cm. Pollen application from 100 cm provided better fruit set in pistachio. In artificial pollination, a lot of pollen grains settled on the stigma surface; for this reason flowers and small fruits were abscised. According to our data, more pollen caused less fruit set in pistachio.

Pistil receptivity as measured by fruit set was highest in experiments in which only one pollen application was performed at the anthesis (0 day) with 5% pollen mixture, and receptivity continued up to 4 days after anthesis. Mean fruit set was highest in experiments that had one pollen application on 0 and 4 days after anthesis, whereas two pollen applications on 0+4 days resulted in the lowest fruit set (Table 2).

Pollen density

The number of pollen grains per unit area in the artificial pollination was counted under a light microscope, and the highest pollen grain number was observed on 100% (pure) pollen applied from 25 cm distance. The lowest pollen grain number was

Table 1 Average fruit set of *Pistacia vera* 'Siirt' pollinated with different pollen and flour mixtures of Male 13 at different application distances (%). (Letters next to numbers indicate different groups determined by Tukey test ($P \leq 0.05$).)

Pollen application distances (cm)	Pollen and flour mixtures					Mean fruit set (%)
	1% pollen	5% pollen	10% pollen	50% pollen	100% pollen	
25	6.55 b-e	8.80 ab	7.94 a-d	8.26 a-d	4.22 f	7.15
50	5.59 ef	9.27 a	6.57 b-e	8.41 abc	6.12 cde	7.19
100	5.68 de	9.51 a	9.26 a	9.24 a	5.23 ef	7.79

obtained from 1% pollen mixture when applied from 50 and 100 cm; 5% and 10% pollen mixture applied from 100 cm (Table 3).

The best pollen mixture for fruit set and the number of pollen grains per mm^2 were used to determine adequate pollen grain numbers to pollinate a flower. High fruit set was generally determined on 5% pollen mixture (Table 1). Fruit set did not increase as soon as the number of pollen grains per unit of stigma area increased. Furthermore, increased number of pollen grains per stigma area would cause excessive flower abscission after a certain point, and this could be the cause of low fruit set in pistachio.

Number of pollen grains per 1 mm^2 was on average 4.60 at 5% pollen mixture (Table 3). According to Kaska et al. (1989), the average stigma surface of pistachio flower was calculated as 3 mm^2. Thus, adequate pollen grain numbers to pollinate a flower was calculated as 14 or 15 pollen grains per flower in pistachio. Acar (2004) reported that an average of 15 pollen grains germinated on stigma, 6.2 pollen tubes reached to the base of style among them, 1.2 pollen tubes found the funiculus, and on average 0.5 pollen tubes reached the embryo sac in 'Siirt'.

More or less than 15 pollen grains per stigma may cause a decrease in fruit set. But stigma receptivity, pollen vigour, and pollen germination capacity may affect fruit set as significantly as other factors.

DISCUSSION

It is hypothesised that germinated pollen grains and pollen tubes absorb water and nutrients from the flowers and weaken the flowers, so the flowers abscise (Acar 2004; Acar & Eti 2007). Low fruit set obtained from pure pollen application supported this hypothesis (Table 1, Fig. 2). According to McGranahan et al. (1994), higher fertilisation and lower fruit abscission has been reported with few pollen grains on the stigma, which may be related to less competition between pollen tubes in the pollen tube pathway. In the pure pollen (100% pollen) application, an average of 16.65 pollen grains were found on the 1 mm^2 microscope slide area (Table 3). Thus, when the pure pollen was applied to the inflorescence, too much pollen came to the stigma surface and simultaneously germinated on the stigma.

In pistachio, final fruit set varied between 9.40% and 16.50% of flowers (Ayfer et al. 1990; Kuru & Ayfer 1990) and depended on pollination, fertilisation, nutrition, irrigation, and pruning (Goldhamer et al. 1988; Arpaci et al. 1995; Tekin et al. 1995). According to Crane & Iwakiri (1981), embryo abortion in pistachio has been variously attributed to lack of pollination, poor nutrition, rainfall during anthesis, and water deficit during seed development. Acar et al. (2001) reported that 9.40% of initial flowers turned

Table 2 Average fruit set of *Pistacia vera* 'Siirt' pollinated with different pollen and flour mixtures of Male 13 at 0, 2, and 4 days after anthesis (%). (Letters next to numbers indicate different groups determined by Tukey test ($P \leq 0.05$).)

Time of pollination	Pollen and flour mixtures					Mean fruit set
(days)	1% pollen	5% pollen	10% pollen	50% pollen	100% pollen	(%)
0	5.37 f-j	11.72 a	10.24 a-e	7.70 a-i	6.25 f-j	8.26
2	7.79 a-i	8.81 a-i	8.58 a-h	7.59 a-i	4.78 hij	7.51
4	6.45 c-i	10.54 abc	7.71 a-i	10.81 abc	5.77 e-j	8.26
0+2	6.82 a-i	7.75 a-i	9.84 a-e	8.18 a-i	5.17 f-j	7.55
0+4	4.42 ij	7.60 a-i	6.43 d-j	9.09 a-g	2.75 j	6.06
2+4	6.32 e-j	9.29 a-e	7.74 b-i	5.75 ghij	4.89 hij	6.80
0+2+4	4.41 hij	8.64 a-g	4.93 hij	11.36 ab	6.71 d-j	7.21

Table 3 Average number of pollen grains per 1 mm^2 area applied in various pollen and flour mixtures with different application distances. (Letters next to numbers indicate different groups determined by Tukey test ($P \leq 0.05$).)

Pollen application	Pollen and flour mixtures					Mean fruit set
distances (cm)	1% pollen	5% pollen	10% pollen	50% pollen	100% pollen	(%)
25	3.53 cd	7.35 bcd	7.34 bcd	16.45 ab	22.33 a	11.40
50	2.35 d	3.53 cd	4.42 cd	15.27 abc	16.76 ab	8.47
100	3.08 d	2.93 d	3.24 d	9.70 bcd	10.87 abcd	5.96

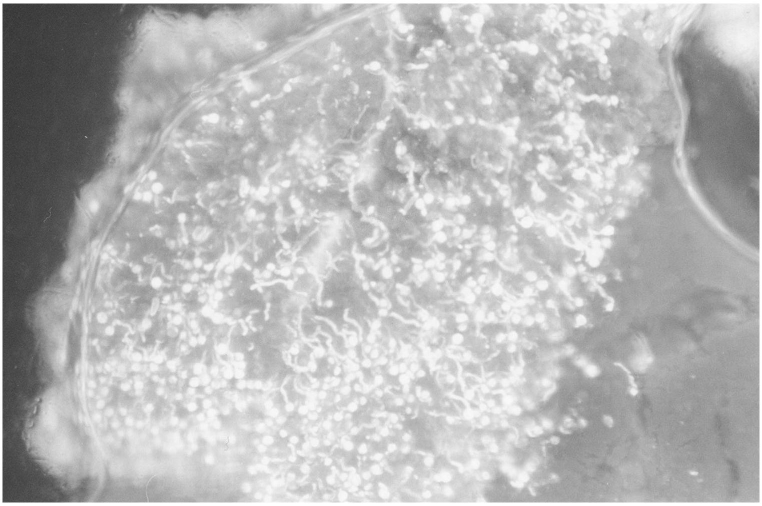

Fig. 2 Number of germinated pollen on the stigma when using pure (100%) pollen (magnification ×63).

to fruit from open pollination in 'Siirt' whereas this was 14.13% in artificial pollination. There are some reasons for flower and fruit abscission. The majority of pollinated pistils of pistachio did not reach maturity and were either shed or resulted in seedless or small-seeded fruits because of embryo sac degeneration (Shuraki & Sedgley 1996). Boron (B) nutrition affects fruit set and yield in pistachio. B applications result in enhanced pollen germination, reduced flower drop, and decreased blank nut percentage (Brown et al. 1994). Few pollen grains on the stigma, which may be related to less competition between pollen tubes in the pollen tube pathway, provide higher fertilisation and lower fruit abscission (Shuraki & Sedgley 1997). Fruit set in pistachio is determined by flower and small fruit abscission seen as cluster shedding and sparsing. Cluster shedding mainly occurs at lower branches, and results inaccumulation of food resources and hormones at the branch tips. 33.3% of flowers died before reaching receptive maturation. Such flowers did not have mother cells and were located within clusters of full blooming stage. In addition, during anthesis of pistachio, 26.7% and 23.3% of flowers were at sporogenesis and gametogenesis stages, respectively. The yield was determined by the developmental stage of the female flowers, and this is affected by rainfed conditions during spring and autumn, soil cultivation, fertilisation, and pruning (Ayfer et al. 1990).

Main flower and fruit abscission occurred during an initial 35 days after full blooming in 'Siirt', and 50 days after full blooming in 'Kirmizi' and 'Ohadi' pistachio. Abscission in 'Siirt' was higher than in 'Ohadi' and 'Kirmizi' (Acar & Eti 2007).

The mixtures of 1%, 5%, and 10% of pollen+flour or pure pollen were sprayed on pistachio trees at the beginning of flowering, and fruit set was found to be high for all pollen applications compared with naturally pollinated trees (Caglar & Kaska 1995). The successful results were obtained from 1% of the pollen+flour mixture for pistachio trees in artificial pollination; and in fruit setting there was no significant difference between one or two pollen applications (Kuru 1995). Similar results have been observed in this study, where pollen mixtures provided ease of artificial pollination because of less pollen usage.

Kuru & Ayfer (1984) reported that there are no important differences between fruit set of one artificial pollination at 1% of flowers opening on the inflorescence and two applications at 1% and 40% of flowers opening in the pistachio trees. Pistachio trees have been pollinated at 0, 1, 2, 3, and 4 days after anthesis with pollen of three different male genotypes, and pollen tube growth and fruit set were highest up to 2 days after anthesis (Shuraki & Sedgley 1994). The results obtained in the study are in agreement with the research of Kuru & Ayfer (1984) and Shuraki & Sedgley (1994).

The results indicate that for optimum fruit production of pistachio, 5% and 50% pollen mixtures should be sprayed from a 100 cm distance at 0 and 4 days after anthesis. Adequate pollen numbers to pollinate a flower was calculated as 14 or 15 pollen grains per flower in pistachio. Increased number of pollen grains per stigma area would cause excessive flower abscission after a certain point, and this could cause low fruit set in pistachio.

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