



Effect of Artificial Pollination on Some Quantitative and Qualitative Traits of Three Pistachio Cultivars in Qazvin Province

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ABSTRACT

This study evaluated the effect of artificial pollination on pistachio trees in an orchard at a low ratio of male to female trees in Qazvin province, Iran, over the years 2012-2013. Experimental factors included three female cultivars (Akbari, Owjadi, Kalebozi) and three types of pollinizer (Control, GA, and GB) with three replications. In each female tree, two branches were selected in the northern and southern directions and bagged before flowering. When stigma was ready to receive pollen, artificial pollination was carried out using the male genotypes GA and GB. The traits of some fertilized flowers per cluster, initial and secondary abscission of flowers and fruits, number of fruits per cluster, nut dry weight per cluster, nut dimension, blankness percentage, and non-split nuts percentage were investigated. The results indicated that artificial pollination in three stages after flowering not only increased the quantitative traits such as number of flowers and fruits per cluster but also reduced the percentage of non-split fruits and blank fruits in all cultivars. The highest and lowest numbers of fertilized flowers were obtained by pollination with GA (91.67) and open pollination (73.89), respectively. Besides, the lowest and highest percentages of initial and secondary abscission of flowers belonged to pollination by GA (13.11-21.44 %) and open pollination (18.33-42.11%), respectively. In addition, the highest initial (54.44) and final (27.66) fruit sets per cluster, the lowest blank nuts (18.01%), and non-split nuts (17.62%) were achieved using pollination with GA. Therefore, it can be concluded that GA is the best male tree for pollination. Akbari cultivar showed the highest nut dry weight per cluster (34.81 g) and the lowest (14.53%) percentage of blank nuts.

Introduction

Iran is the largest producer of pistachio among the major pistachio producers in the world. However, the yield of pistachio in Iran (1300 kg/ha) is lower than the main

pistachio-producing countries (FAO, 2017). Therefore, it is necessary to improve the quantity and quality of pistachio in Iran (Eslami *et al.*, 2019). One of the major

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factors that affect the quality of pistachio nuts is presence of sufficient male trees and appropriate pollination (Sharifkhah *et al.*, 2020). The production of blank nuts mainly results from insufficient pollination, fertilization failure, spring rains during blooming, nutrient deficiency, genetics, and water and salt stress during the kernel development (Crane and Iwakiri, 1985; Ferguson *et al.*, 2005; Alipour, 2018; Tajabadipour *et al.*, 2018; Norozi *et al.*, 2019; Akca *et al.*, 2020). Most male pistachio trees tend to flower sooner than female trees and usually release their pollen when the female flowers are not ready to receive pollen grain (Ak *et al.*, 1996). Therefore, female flowers are usually unable to receive enough pollen grains (Ozeker *et al.*, 2006). In most orchards, male trees are not planted at a proper ratio and in an appropriate direction. Furthermore, pollen quality is low in most orchards (Zeraatkar *et al.*, 2003). Insufficient pollination reduces tree crop and increases the chances of blank nut formation (Isfendiyaroglu *et al.*, 2001; Zeraatkar, *et al.*, 2003). Pontikis (1989) found that unsuccessful pollination in pistachio cultivar *P. vera* occurred due to the failure of female and male trees to flower simultaneously, leading to the non-overlapping between pollen release of male flowers and pollen reception of female flowers.

When natural pollination is defective or pollen does not reach the female tree at the proper time, pollination can be applied using supplementary or artificial pollination by spraying pollen over the trees manually or using a spray device (Izadi and Aslmoshtagh, 2014; Sharifkhah *et al.*, 2020). The effectiveness of artificial pollination depends on environmental factors such as wind and rainfall. Artificial pollination is considered as a temporary solution when natural pollination is inadequate (Acar *et al.*, 2001) and proven to be effective in some countries such as Iran, Syria, and Tunisia, (Caglar and Kaşka, 1994; Zeraatkar *et al.*, 2003).

Abu-Zahra and Al-Abbadi (2007) used a mixture of pollen grains and an inactive substance (wheat flour) for manual pollination of pistachio trees and concluded that this method was an effective treatment. Isfendiyaroglu *et al.* (2001) investigated the effect of pollen of different pistachio varieties on female cultivars (Kırmızı and Uzun) and concluded that *P. vera* pollen was preferable, because it facilitated higher split and larger nuts. The findings of Riazi and Rahemi (1995) confirmed the role of xenia and meta-xenia phenomena in pistachio fertility because pollen grains caused a difference between kernel growth and other fruit characteristics. They found that larger kernel and higher shell splitting obtained from the use of *P. vera* pollen and *Pistacia* species pollen were undesirable. Therefore, it can be concluded that choosing a suitable pollinizer tree is essential to establishing a pistachio orchard. In this research, in order to identify suitable pollinizer cultivars, the effects of artificial pollination on some quantitative and qualitative traits of three commercial pistachio cultivars in Qazvin province were investigated.

Materials and Methods

In this study the experiments were conducted in a pistachio orchard in Buin-Zahra city, located in the southeastern part of Qazvin province over the years 2012-2013. Factorial experiment with a Randomized Complete Block Design (RCBD) was used with two factors. Experimental factors included three female cultivars (Akbari, Owhadi, Kalebozi) and three types of pollinizer (Control, GA and GB) with three replications in two years.

Female cultivars comprised 15-year-old trees under the same management conditions and GA and GB were local pollinizers. Prominent features of the two selected male parents (GA and GB) included long flowering period and abundance of catkins, making them viable options as pollinizers. In controlled pollination treatments, two branches of every female tree in the

northern and southern directions were selected and bagged before blooming. This action was taken to isolate the flowers and prevent the entrance of any unwanted pollen. In the open pollination treatment, no bagging was performed. When stigma was ready to receive pollen, artificial pollination was carried out using pollen of GA and GB genotypes. Flower clusters of male genotypes were collected before pollen dehiscence, taken into the laboratory, and put in glass vials filled with water and left for one night (Kardoush *et al.*, 2009); then, the anthers were sieved, put in containers tightly interspersed with parafilm, and kept in a refrigerator at 3-4°C until female trees flowered. Some calcium chloride was also placed inside the containers to absorb moisture. Manual pollination was performed in three stages after flowering by a hand brush. Ten days after full bloom, the bags were removed and fertilized and abscised flowers per cluster were counted on the cluster. In addition, the initial and final set nuts, nut dry weight per cluster, nut length, nut width, nut thickness, blankness percentage, and non-split fruit percentage were investigated. Initial and secondary flower drops

were calculated 10 and 20 days after full bloom, respectively. Initial and final fruit sets per cluster were recorded 25 days after full bloom and at harvest time, respectively. Data analysis was conducted using MSTATC software and a comparison of the means of data was done using Duncan's test.

Results

The results of analysis of variance showed the number of fertilized flowers per cluster, initial and secondary flower abscission, initial fruit set, nut dry weight per cluster, nut length, nut width, and percentage of blankness and non-splitting were affected by female cultivars ($P \leq 0.01$), while the number of final fruit set and nut thickness remained unaffected ($P \leq 0.05$). The effect of pollinizer on all of the above traits was also significant ($P \leq 0.01$), except for nut dry weight per cluster, nut length, nut width, and nut thickness ($P \leq 0.05$). The effect of interaction between female and male parents was significant only on fertilized flowers per cluster and initial fruit sets ($P \leq 0.01$) and the other traits were not affected ($P \leq 0.05$) (Table 1).

Table 1. Analysis of variance of some traits of fruits taken from artificially pollinated trees

SOV	Df	Fertilized flowers per cluster	Initial abscission	Secondary abscission	Initial fruit set per cluster	Final fruit set per cluster	Nut Dry weight per cluster	Nut length	Nut width	Nut thickness	Blankness	Non-splitting
Cultivar(A)	2	160.8**	34.4**	53.8**	61.7**	6.259 ^{ns}	855.52**	6.663**	1.067**	0.039 ^{ns}	424.2**	159.7**
Pollinizer (B)	2	732.4**	62.4**	1103.3**	2536.4**	96.704**	3.243 ^{ns}	0.047 ^{ns}	0.136 ^{ns}	0.07 ^{ns}	121.5**	134.3**
A×B	4	13.8**	0.2 ^{ns}	1.75 ^{ns}	0.88**	5.426 ^{ns}	4.458 ^{ns}	0.133 ^{ns}	0.135 ^{ns}	0.029 ^{ns}	1.55 ^{ns}	3.3 ^{ns}
Error	18	2.19	1	1.13	2.02	2.815	4.599	0.165	0.058	0.036	1.1	0.95

ns: not significant, **: p-value <0.01. *: p-value <0.05. Initial abscission of flower: 10 days after full bloom; secondary abscission of flower: 20 days after full bloom. Initial fruit set per cluster: 25 days after full bloom; final fruit set: at harvest time

Fertilized flowers per cluster

The results of analysis of variance (Table 1) showed that the number of fertilized flowers per cluster was significantly affected by cultivar and pollinizer ($P \leq 0.01$). Based on the comparison of the means of data, Akbari cultivar with 88 flowers per cluster had the highest fertilized flowers, followed by Owhadi (83.44)

and Kalebozi (79.56) cultivars (Table 2). Pollinizers GA and GB showed significantly different results from the control. The highest and lowest numbers of fertilized flowers were obtained using pollination with GA (91.67) and open pollination or control (73.89), respectively. The highest number of fertilized flowers

(90.67) per cluster was observed with the application of Akbari × GA combination (Fig. 1).

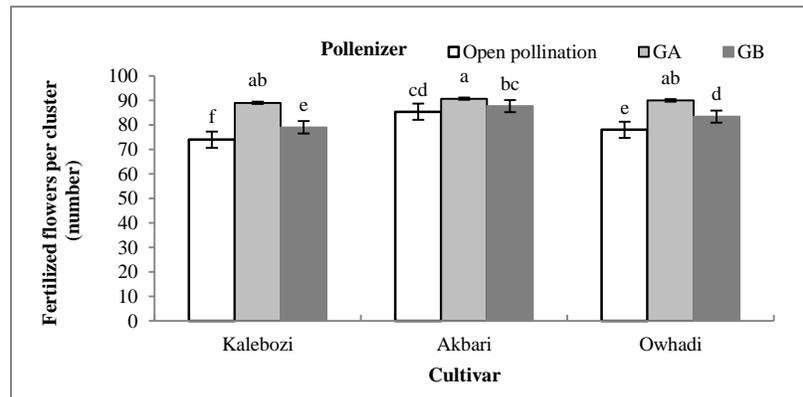


Fig. 1. Effect of interaction between male and female cultivars on fertilized flowers per cluster

Initial and secondary flower abscission

The results of analysis of variance (Table 1) showed that the traits of initial (10 days after full bloom) and secondary (20 days after full bloom) flower drops were significantly affected by cultivars and pollinizers ($P \leq 0.01$), but they were not affected by the interaction between cultivar and pollinizer ($P \leq 0.05$).

Based on the comparison of the means of data, Akbari cultivar with 13.44-27 (initial-secondary) had the lowest abscission rate of all cultivars including Owahdi (15.78-29.56) and Kalebozi (17.34-31.89) (Table 2). Pollinizer type had significant effects on the initial and secondary drops of the female flowers. Thus, the lowest number of initial and secondary abscission of flowers (13.11-21.44) was obtained using hand pollination by GA and the highest number (18.33-42.11) using open pollination (Table 2).

Initial and final fruit sets

The results of analysis of variance showed that the effect of cultivar, pollinizer, and the interaction between

the two on the initial fruit set per cluster (25 days after full bloom) was significant ($P \leq 0.01$); however, the final fruit set (at harvest time) was only affected by pollinizer ($P \leq 0.01$) and not affected by female cultivars and the interaction of female and male parents ($P \leq 0.05$). Means comparison of the data (Table 2) showed that the highest initial fruits per cluster (46.44) belonged to Akbari, followed by Owahdi (42.89) and Kalebozi (41.33). Among pollinizers, the highest initial fruit set per cluster was also obtained using pollination with GA (54.44), which showed a significant divergence from the pollinizer GB (52) and open pollination (24).

The final fruit set was affected by pollinizer at harvest time. Therefore, the highest final fruit set per cluster was also obtained by pollination with GA (27.66), followed by GB (24.33) and open pollination (21.11). Female cultivars did not show significant differences in terms of the number of final fruits. The highest initial fruit set per cluster (54.66) was observed in the Kalebozi × GA combination (Fig. 2).

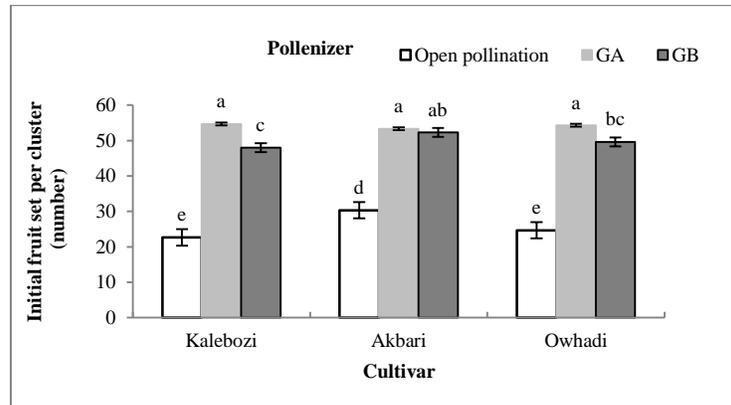


Fig. 2. Effect of male and female cultivar interaction on initial fruit set

Nut dry weight per cluster

The results of analysis of variance (Table 1) showed that the effect of female cultivar on nut dry weight per cluster was significant ($P \leq 0.01$). However, nut dry weight per cluster was not affected by pollinizer and the interaction of female cultivar and pollinizer ($P \leq 0.05$).

Means comparison of the data showed that the highest nut dry weight per cluster (34.81 g) belonged to Akbari, followed by Owhadi (19 g) and Kalebozi (17.02) cultivars (Table 2). As mentioned earlier, pollinizer type did not have significant effect on nut dry weight per cluster (Table 3).

Fruit Dimension

The results of analysis of variance (Table 1) showed that length and width of nut were significantly affected by the female cultivar ($P \leq 0.01$), but nut thickness was not affected by the female cultivar ($P \leq 0.05$). The effect of pollinizer and interaction of cultivar and pollinizer on length, width, and thickness of nut was not significant ($P \leq 0.05$).

Means comparison of the data showed that the highest nut length (18.97 mm) belonged to Akbari cultivar, followed by Owhadi (17.61 mm) and Kalebozi (17.38 mm) cultivars. The highest nut width (12.51 mm) was observed in Kalebozi, followed by Akbari (12.51 mm) and Owhadi (11.88 mm) cultivars, although the

difference between Kalebozi and Akbari cultivars was not significant (Table 2).

Blank nuts

According to the results of analysis of variance (Table 1), the percentage of blank nuts per tree was significantly affected by the cultivar and pollinizer ($P \leq 0.01$). However, the interaction effect of cultivar and pollinizer was not significant on this trait ($P \leq 0.05$). The comparison of the means of data showed that Akbari cultivar produced the lowest percentage (14.53%) of blank nuts. Kalebozi cultivar yielded the highest percentage of blank nuts (28.22%) (Table 2). The effect of pollinizer on the percentage of blank nuts was also significant. The lowest and highest percentages of blank nuts belonged to pollination with cultivar GA (18.01%) and open pollination (25.14%), respectively (Table 3).

Non-Split nut percentage

The results of analysis of variance (Table 1) showed that the percentage of non-split nuts as an undesirable trait was significantly affected by the cultivar and pollinizer ($P \leq 0.01$). However, the effect of the interaction between cultivar and pollinizer on this trait was not significant ($P \leq 0.05$). Based on the mean comparison of the data (Table 2), the percentage of non-

splitting nuts in Akbari cultivar (15.13%) was lower than those in Owhadi (20.17%) and Kalebozi (23.5%) cultivars. Furthermore, the highest percentage of non-split nuts was obtained using open pollination (24.05%)

treatment, which was significantly different from the percentage obtained by other pollinizers. The difference between GA and GB was not statically significant (Table 3).

Table 2. Comparison of the means of traits with different pistachio cultivars

Cultivar	Fertilized flowers per cluster (number)	Initial abscission of flower (%)	Secondary abscission of flower (%)	Initial fruit set per cluster (number)	Final fruit set per cluster	Dry weight of fruit per cluster (g)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Blankness (%)	Non-splitting (%)
Kalebozi	79.56 ^c	17.34 ^a	31.89 ^a	41.33 ^c	23.55 a	17.02 b	17.38 ^b	12.51 ^a	11.41 a	28.22 ^a	23.5 ^a
Akbari	88 ^a	13.44 ^c	27 ^c	46.44 ^a	25.22 a	34.81 a	18.97 ^a	12.45 ^a	11.28 a	14.53 ^c	15.13 ^c
Owhadi	83.44 ^b	15.78 ^b	29.56 ^b	42.89 ^b	24.33 a	19 b	17.61 ^b	11.88 ^b	11.37 a	20.44 ^b	20.17 ^b

Initial abscission of flower: 10 days after full bloom; secondary abscission of flower: 20 days after full bloom; Initial fruit set per cluster: 25 days after full bloom; final fruit set: harvest time

Table 3. Comparison of the means of traits with different pistachio pollinizers

Pollinizer	Fertilized flowers per cluster (number)	Initial abscission of flower (%)	Secondary abscission of flower (%)	Initial fruit set per cluster (number)	Final fruit set per cluster	Dry weight of fruit per cluster (g)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)	Blankness (%)	Non-splitting (%)
GA	91.67 a	13.11 c	21.44 c	54.44 a	27.66 a	24.02 a	17.98 a	12.15 a	11.32 a	18.01 c	17.62 b
GB	85.44 b	15.11 b	24.89 b	52.0 b	24.33 b	23.88 a	18.06 a	12.4 a	11.45 a	20.04 b	17.13 b
Open pollination	73.89 c	18.33 a	42.11 a	24.0 c	21.11 c	22.92 a	17.92 a	12.3 a	11.28 a	25.14 a	24.05 a

*Means with the same letter are not significantly different at p=0.05 using Duncan's multiple range test.

Initial abscission of flower: 10 days after full bloom; Secondary abscission of flower: 20 days after full bloom; Initial fruit set per cluster: 25 days after full bloom; final fruit set: harvest time

Discussion

Pistachio trees produce abundant flowers. Lack of pollination, type of pollinizer parents, and environmental and nutritional conditions affect flower and fruit drops (Stephenson, 1981; Acar and Eti, 2007; Khezri et al., 2010). Various studies have shown that pollen grains of species other than *P. vera* have undesirable effects on the development of embryonic sac (endosperm and embryo) and kernel weight and increase the probability of blankness and fruit abscission.

A significant level of abscission occurs following pollination and fruit setting. Kalebozi and Akbari cultivars experienced the highest and the lowest flower

and small fruit abscission rates, respectively. Ten days after full bloom, the cultivars pollinated by male GA produced a larger number of fertilized flowers than the ones pollinated by GB and control.

The percentage of flowers and fruits dropping from the trees on the 20th day after full bloom was higher than initial abscission (10 days after full bloom). Acar and Eti (2007) found that the major flower and fruit abscission occurred in the initial 35 days after full bloom in Siirt and 50 days after full bloom in the pistachio cultivars of Kirmizi and Owhadi.

In the case of pistachio, a majority of fertilized flowers are abscised or do not reach maturity due to

embryo sac degeneration and production of seedless or small seeded fruits (Shuraki and Sedgley, 1996). Therefore, the final fruit set for pistachio changes between 9.40-16.50% (Ayfer *et al.*, 1990; Kuru and Ayfer, 1990; Acar *et al.*, 2001). Ayfer *et al.* (1990) reported 80-130 flowers per cluster and reported 83.5% abscission.

As mentioned earlier, while the initial fruit set per cluster (25 days after full bloom) was significantly affected ($P \leq 0.01$) by the effect of cultivar, pollinizer, and the interaction between the two, the final fruit set (at harvest time) was only affected by pollinizer ($P \leq 0.01$). Following pollination by male GA, the yield of initial fruit set increased in comparison with GB and open pollination. Kardoush *et al.* (2009) evaluated the effect of pollen of different pistachio species on three pistachio cultivars. Their results showed that *P.atlantica* (male) \times Ashouri (female) combination produced a higher percentage of fruit set (79.9%) after 15 days of pollination. Moreover, the effect of source of pollen grain on fruit set and quality was reported in other crops e.g. plum (*Prunus salicina* Lindl.) (Barzamini and Fotouhi Ghazvini, 2017.)

Nut dry weight was only affected by female cultivars. The highest nut dry weight per cluster (34.81 g) was recorded in Akbari cultivar, followed by Owhadi (19 g) and Kalebozi (17.02) cultivars. Acar *et al.* (2001) reported that artificial pollination was found effective in *P. vera* cv. Siirt and could be applied to pistachio orchards. In the present research, the largest nuts were found in Akbari cultivar, while the effect of pollinizer and the interaction between cultivar and pollinizer on nut dimension was not significant ($P \leq 0.05$). Crane and Iwakiri (1980) reported that using *P. vera* pollen increased kernel size compared to the pollen of other species. Kardoush *et al.* (2009) found that artificial pollination improved fruit dry weight and nut size of pistachio, compared to open pollination. Based on their results, a combination of *P.khinjuk* as a male parent and

Batouri as a female cultivar produced the maximum fruit length, width, and thickness, compared with open pollination.

Akbari and Kalebozi cultivars produced the lowest (14.53%) and highest (28.22%) percentages of blank nuts, respectively. Among pollinizers, the lowest and highest percentages of blank nuts were produced using GA (18.01%) and open pollination (25.14%), respectively.

It has been reported that the blank nuts ratio is affected by genetic and culture-based operations such as irrigation and nutrition, environmental conditions, pollination efficiency, and rootstock (Ozeker *et al.*, 2005; Kaska, 1995). Low percentages of blank nuts produced using GA pollen resulted from their different genetic structures. In experiments conducted on California commercial pistachio, electrostatic pollination has led to increase in the percentage of fruit set and decrease in the percentage of blank nuts (Vaknin *et al.*, 2002). Acar *et al.* (2001) reported that the percentages of blankness of pistachio nuts produced were 47.76% and 55.32% in manual control pollination (artificial) and open pollination, respectively. Ozeker *et al.* (2005) found that the pistachio nuts treated by pollination with *P. vera* pollen generated lower blankness than other pollinizers (*P. atlantica* and *P. terebinthus*). According to the findings of Kardoush *et al.* (2009), the highest and lowest mean percentages of blank nuts grown in Ashouri and Batouri cultivars were 50.9 and 40.5%, respectively.

The percentage of split nuts is an important feature that helps one select a proper pistachio cultivar. As reported by a number of studies, splitting is affected by the cultivar, pollinizer (Ozeker *et al.*, 2005), and water supply during kernel growth (Kaska, 1990). There are reports that are indicating that, using artificial pollination increased the number of split nuts or, in other words, decreased the number of non-split nuts. Ozeker *et al.* (2005) reported that the highest and lowest

percentages of splitting were 30.45 % by *P. vera* pollination and 22.10 % by *P. atlantica* pollination, respectively. Crane and Iwakiri (1980) found that the use of pollen of *P. vera* increased split nuts and kernel growth in comparison with the pollen of *P. atlantica* and *P. terebinthus* species. Ka-ka and AK (1996) reported that *P. vera* pollen caused a higher percentage of split nuts than pollen of *P. terebinthus* and *P. atlantica*. Another related study has investigated the effect of pollen of *P. vera* (Peters and Ask cultivars), *P. atlantica*, and *P. terebinthus* on Kerman cultivar and the obtained results have shown that *P. vera* as a male parent can be an appropriate choice to achieve the highest percentage of split nuts. Considering the effect of male cultivars on the degree of nut splitting, selecting an appropriate pollinizer tree is important.

Conclusions

The purpose of this study was to select an appropriate pollinizer for manual control of pollination of three cultivars of Akbari, Owjadi, and Kalebozi in Qazvin province, Iran. The results showed that manual control of pollination not only increased the number of fertilized flowers and fruit set per cluster but also reduced the number of flower abscission, blankness percentage, and non-split nuts of pistachio cultivars. Nut dry weight per cluster and nut dimension were not affected by pollinizer. These results were obtained using viable data on the genetic characteristics of female cultivars and pollinizers. Based on the results, the best male tree was GA treated, which was found to be significantly different from open pollination treatment. Therefore, GA can be used in pollination to ensure high-quality fruit production and a higher fruit set percentage. Open pollination yielded the highest blank nuts.

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