

Determining the Pollinizer for Pecan Cultivars

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Abstract

This study was conducted to determine the best pollinizer for five selected pecan cultivars in southwest of Iran at Safiabad Agricultural Research Center in 2014-2015. The cultivars included: 'GraTex', '10J', 'Wichita 6J', 'GraKing', 'Choctaw' as pollinated cultivars (♀) and 'GraTex', 'Peruque', 'Comanche 4M', '10J', 'Wichita 6J', 'Mohawk', 'Mahan', 'Stuart 2J', '3J', 'Stuart 4J', 'GraKing', 'Choctaw', 'Apache', '6M', 'Wichita 7J' and 'Comanche 5M.' as pollinizer cultivars (♂). In the first step, a pollination chart of cultivars was determined in two years. The pollination chart of cultivars showed that all the cultivars investigated during this study were dichogamous and also protogynous except for the 'Peruque'. 'GraKing' had the longest duration of shedding pollen. Pollination chart showed that 'Peruque', 'GraKing', and 'Stuart 2J' had flowering overlap with the selected cultivars. Pollen germination test showed that the germination ability was different among the cultivars. It was 45% for 'GraKing' and 35% for 'Peruque', which were both recommended as pollinizers in this study. '6M', 'GraTex' and 'Stuart 4J' cultivars had the highest pollen germination percentage of 65%, 60% and 60%, respectively. The results of controlled pollination test showed that different pollen sources had no significant effect on nuts per cluster but self-pollinated all of the cultivars significantly reduced fruit set in first and second years. Based on the present research, pollination in pecan orchard was necessary for adequate yield. Also, 'Peruque', 'GraKing' and 'Stuart 2J' were the best pollinizers for five selected cultivars in southwest of Iran.

Keywords: *Carya illinoensis*, Controlled pollination, Pollen germination, Pollination chart.

Introduction

Pecan [*Carya illinoensis* (Wagenh.) K. Koch] is a monoecious crop. Male flowers (staminate) were organized into a catkin at the end of the last season's growth, and female flowers (pistillate) were born on a spike at the end of the current season's growth (Wetzstein and Sparks, 1986). A single catkin can produce as many as 2.64 million pollen grains. Only one pollen grain is required to produce one pecan (Wells, 2007).

Pollination in the pecan orchard is critical to both the yield and quality of nuts (Conner, 2007). Pecan cultivars differ with respect to maturity of the staminate and pistillate flowers, leading to both protandrous and

protogynous patterns of flowering, termed heterodichogamy. In the protogynous type, the pistils mature first before the stamens shed pollen.

In the protandrous type, the opposite is true with stamens shedding pollen before pistils mature. With the separation of stigma receptivity and pollen shed, self-pollination is limited (Wood, 1997. Wood, 2000 and Wells, 2007)

Cross-pollinated pecans are usually larger and have a higher quality than self-pollinated pecans (Conner, 2007). Lack of pollinizers can cause an increase in self-pollination, which reduces nut set and nut quality

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(Conner, 2007). Pollination appears to play a role in fruit drop and final nuts per cluster. The major nuts drop section in pecan occurs when trees were self-pollinated (Wood, 2000). Thus, fruit drop from about 2 to 7 weeks after pollination was likely due to problems with pollen availability or self-pollination (Well, 2007).

Suitable pollinizer cultivars within the orchard are mandatory for optimal production. The first step to ensure adequate pollination is to determine a pollination chart. These charts list the pollen shed and pistil receptivity times of the main cultivars. It is recommended to find at least two to three cultivars that shed pollen at the same time the main cultivar is receptive (Conner, 2007).

In order to obtain the true germination ability of pollen grains, the compounds used in pollen grains germination medium must be very similar to the key chemical compounds of stigma (Conner, 2011). Various factors affect the *in vitro* pollen germination, including the concentration of Ca^{2+} , H^+ and B^{3+} ions. Polyethylene glycol (PEG) is used to reduce the water potential in culture medium (Conner, 2011). Percent germination of fresh pollen was variable, with 'Mohawk', 'Wichita' and 'Curtis' germinating at 72%, 64% and 51%, respectively (Janick and Moore, 1996).

Self-pollination of pecan can reduce crop yield by as much as 75% (Conner, 2007) and all of cultivars reduced nut set (Wood, 1997, Wood, 2000 and Wells, 2007).

The objective of this research was to determine the best suitable pollinizers for five selected pecan cultivars (Ajamgard *et al.*, 2013) and their pollination chart. Then, we obtained the true germination ability of pollen grains. Finally, the influence of pollinizer cultivars on nut set in pollinated cultivars was evaluated.

Materials and Methods

This study was conducted at Safiabad Agricultural Research Center (Latitude 32° 16' N, Longitude 48° 25' E, Elevation 82.9m) in 2014-2015. The studied cultivars included 'GraTex', 'Peruque', 'Comanche 4M', '10J', 'Wichita 6J', 'Mohawk', 'Mahan', 'Stuart 2J', '3J', 'Stuart 4J', 'GraKing', 'Choctaw', 'Apache', '6M', 'Wichita 7J' and 'Comanche 5M'.

Determining pollination chart of pecan cultivars

Pecan cultivars were evaluated for the time of staminate maturity (pollen shed), the time of pistillate maturity (stigma receptivity), the end of pollen shed and stigma receptivity.

The time of pollen shed

It is when the first pollen grains will be released by tapping the catkins gently.

The time of stigma receptivity

It is when the color of the surface of stigma changes from a shiny appearance to an almost dark one and secretions are visible at the surface of stigma.

Pollen shed duration

The number of days since the shed of the first pollen grains from the parts that receive sun rays at the south of tree crown until when the last catkins at the northern parts of the tree have been matured and shed their pollen grains.

Stigma receptivity duration

The longevity of female flower with stigma perceptible existed in the tree to be fertilized.

According to these details, the pollination charts of deferent pecan cultivars were determined.

In vitro germination tests of pollen grains

Catkins of each cultivar were collected when 20% of their anthers were matured and dried for 24 hours on a white paper at room temperature of 25°C. Pollen grains released from the anthers were then collected and stored in glass containers at -20°C up until the time of experiment (Conner, 2011).

The culture medium was prepared according to the method of Conner (2011). The culture medium included 5% of sucrose, 20% of polyethylene glycol (PEG), 0.05% of calcium nitrate, 0.025% of citric acid and 10 ml of 2-(*N*-morpholino) ethane sulfonic acid (MES) with an adjusted pH of about 6.0.

Pollen grains were cultivated on layers of cellulosic papers moistened by the compounds used in the culture

medium. A booklet of filter paper having seven 5×5 cm layers was prepared. A sterilized coverslip was placed between layers 6 and 7. The entire booklets including the coverslip were immersed in a vessel containing the culture medium. The cellulose booklets were hanged using forceps to remove the excess medium and then was placed into a closed petri dish to prevent drying (Conner, 2011).

Pollen grains were removed from freezer and were placed into a -4°C refrigerator for four hours. They were then placed above a vessel containing a saturated solution of copper sulfate in order to be hydrated at 25°C for four hours (Conner, 2011). The last layer of cellulose block was edged out from the coverslip, and pollen grains were dispersed on the wet surface of the coverslip by using a fine brush. The door of the petri dish was inserted immediately and the vessels were stored at 25°C for 24 hours.

The coverslip was removed from the cellulose booklets 24 hours after the cultivation of pollen grains. It was then placed on a slide and pollen grains' germination status was evaluated using a microscope with a magnification of 1000×. Each slide was counted four times under the microscope and the average number of replicates was finally recorded. Those pollen grains grown at least as their diameter were considered as germinated (Conner, 2011).

Controlled Pollination

Controlled pollination experiments for every selected cultivar were carried out with complete randomized design with three replications. Pollinated cultivars (♀) include 'GraKing', 'GraTex', 'Wichita 6J', '10J' and 'Choctaw' and pollinizer cultivars (♂) including 'Peruque', 'GraKing', '10J' and 'Mohawk'. For every selected cultivar, two pollinizer cultivars with flowering overlap were selected. In every cross pollination treatments, 25 clusters were selected three days before stigma receptivity were covered by bags. Hand pollination was carried out at time of stigma receptivity by pollinizers and clusters were covered again for seven days.

Nut number per clusters was recorded at two, seven and nine weeks after pollination. Statistical analyses were conducted using SAS software (SAS Institute, Cary, NC,

USA), and means were compared using Duncan's New Multiple Range Test at $P \leq 0.05$.

Results

Determine of pollination chart of pecan cultivars

The results showed that the time of staminate maturity and pollen shed was very variable among different cultivars. Pollen grains began to release in 'Peruque' since April 12th while the staminate maturity and pollen shed happened in 'GraTex' on April 27th.

'Choctaw' and '3J' were the first cultivars ready to receive the pollen grains since April 14th. The study of flowering showed that stigmas in different cultivars were ready to receive pollen grains at approximately 21-26 days after the bud break.

The duration of pollen shed in most cultivars was between 6-10 days except the 'GraKing'. This cultivar had pollen shed duration from April 18th to May 4th for 17 days. The size and number of catkins in this cultivar were different than the other cultivars.

Cultivars did not show any difference for longevity of stigma receptivity. The duration of stigma receptivity in all cultivars was 5-6 days. The surface of stigmas, during this period were exposed to the environmental conditions and dried.

Fig. 1 shows the pollination chart in different cultivars. Results showed that only 'Peruque' was a protandrous cultivar and others were protogynous. The level of dichogamy, however, was not the same for all the cultivars. 'GraTex', 'Comanche 5M', 'Choctaw', 'Apache' and 'Stuart 4J' were completely protogynous, while other cultivars had a pollination overlap between one to four days. First and second pollinizer recommends for selected cultivars were shown in Table 1.

In vitro germination tests of pollen grains

The results showed that pollen grains germination ability in pecan cultivars were significantly different. It was 45% for 'GraKing' and 35% for 'Peruque' of which both were recommended as pollinizers. '6M', 'GraTex' and 'Stuart 4J' cultivars had the highest germination percentage of 65%, 60% and 60%, respectively (Fig. 2). Fig. 3 shows the germination of pollen grains in pecan.

Table 1. Recommended pollinizer for 5 selected cultivars of pecan orchard in southwest of Iran

Main cultivars of pecan	Pecan pollinizers	
	First recommend	Second recommend
'GraTex'	'Graking' + '10J'	'Graking' + 'Comanche 4M'
'Choctaw'	'Peruque' + 'Graking'	'Peruque' + 'Stuart 2J'
'Wichita 6J'	'Peruque' + 'Graking'	'Peruque' + 'Stuart 2J'
'GraKing'	'Peruque' + 'Stuart 2J'	'Peruque' + 'Mohawk'
'10J'	'Peruque' + 'Mohawk'	'Peruque' + 'Stuart 2J'

Controlled pollination

Results of variance analysis showed that the effect of pollen source was significant at 1% level for the number of nuts per clusters. The analysis of pollen source on final

nuts per cluster at nine weeks after hand pollination (First and second years) was shown in Table 2.

Table 2. Effect of pollen sources on final nuts per cluster at 9 weeks after hand pollination (First and second years).

S O V	df	MS									
		'GraTex'		'Choctaw'		'Wichita 6J'		'GraKing'		'10J'	
		First year	Second year	First year	Second year	First year	Second year	First year	Second year	First year	Second year
Pollen source	2	48.2**	39.1**	42.9**	40.0**	51.8**	43.9**	40.7**	41.4**	41.6**	42.3**
Error	6	7.01	6.89	6.19	6.03	7.48	6.98	7.00	7.09	6.82	6.75

**Significant different at 1% level

Discussion

In conclusion, the results showed that both the 'Peruque' and 'GraKing' cultivars could be the pollinizers for other cultivars, especially 'GraKing' with its long period of pollen shedding.

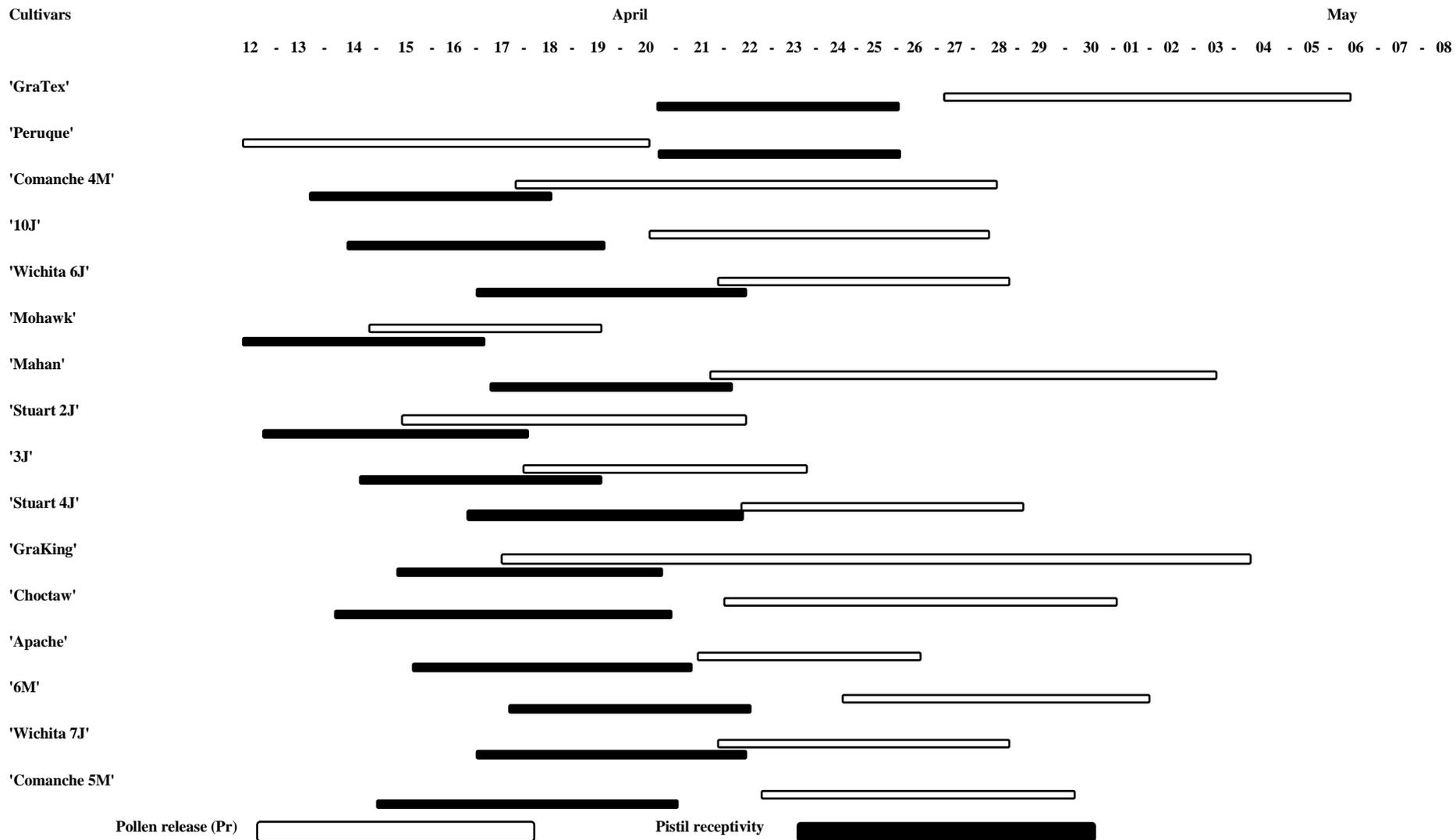
Self-pollination of different pecan cultivars significantly reduced number of nuts per clusters. The major nuts drop section in pecan occurs when trees were self-pollinated (Wood, 2000). Thus, fruit drop from about two to seven weeks after pollination was likely due to problems with pollen availability or to self-pollination (Well, 2007). Self-pollination can reduce crop yield by as much as 75% (Conner, 2007).

Fig. 4 shows means nut per cluster in two, seven and

nine weeks after cross pollination in five selected pecan cultivars. The results indicated that different pollen sources had no significant effect on the number of nuts per clusters. However, self- pollination were significantly reduced fruit set, as also seen by Wood, 2000 and Wells, 2007.

Based on the present study, pollination in pecan orchard is critical and necessary for producing adequate yield. 'GraKing' and 'Peruque' were the best pollinizers among the five selected cultivars. Pollen shedding times of these cultivars overlapped with initiation and duration of pistil receptivity of all selected pecan cultivars in southwest of Iran.

Fig. 1. Pollination chart for pecan cultivars studied at Safiabab Agricultural Research Center of Dezful



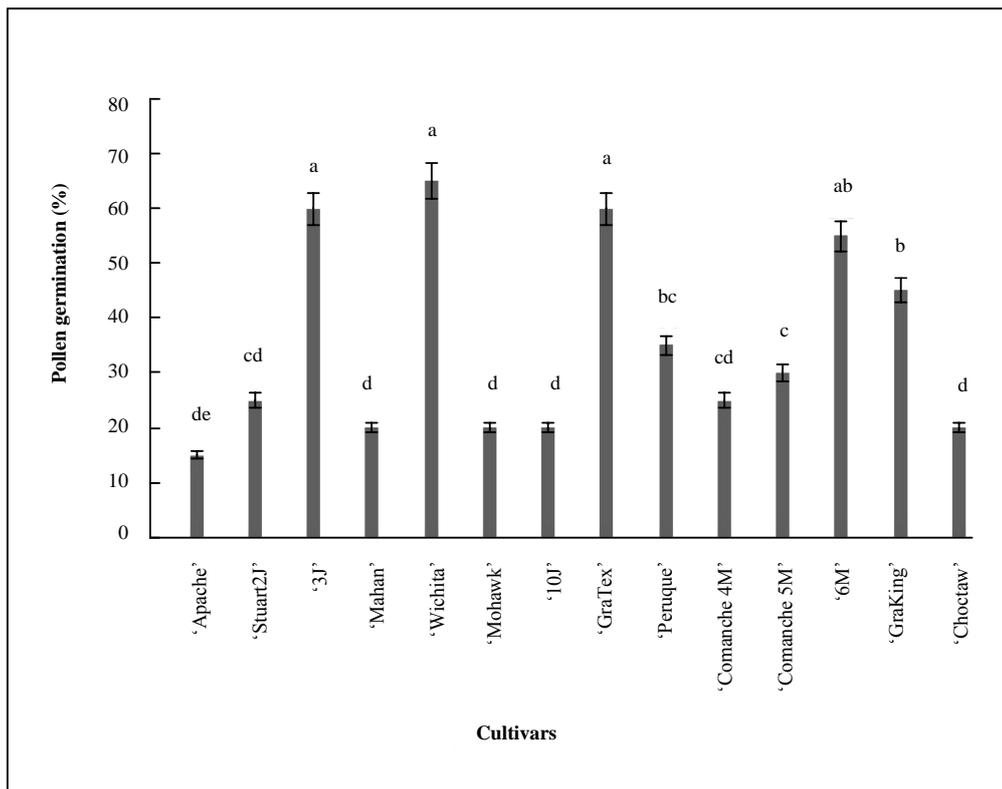


Fig. 2. Pollen germination percentage of different pecan cultivars. Means of columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).



Fig. 3. Pollen grains grown at least as their diameter were considered as germinated (a). Germinated pollen grains of 'GraTex' were showed (b).

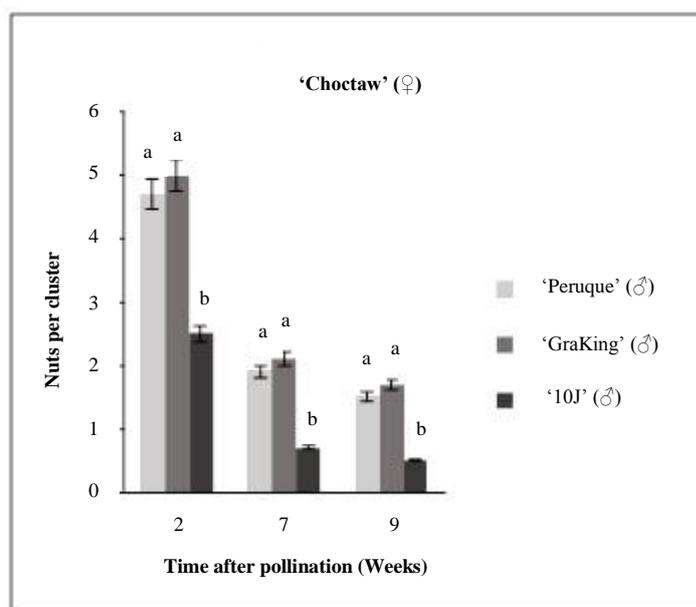
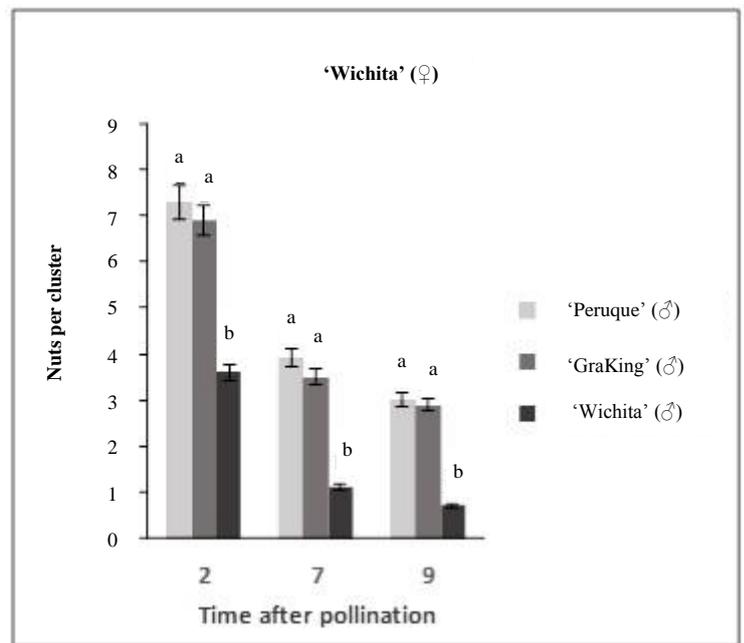
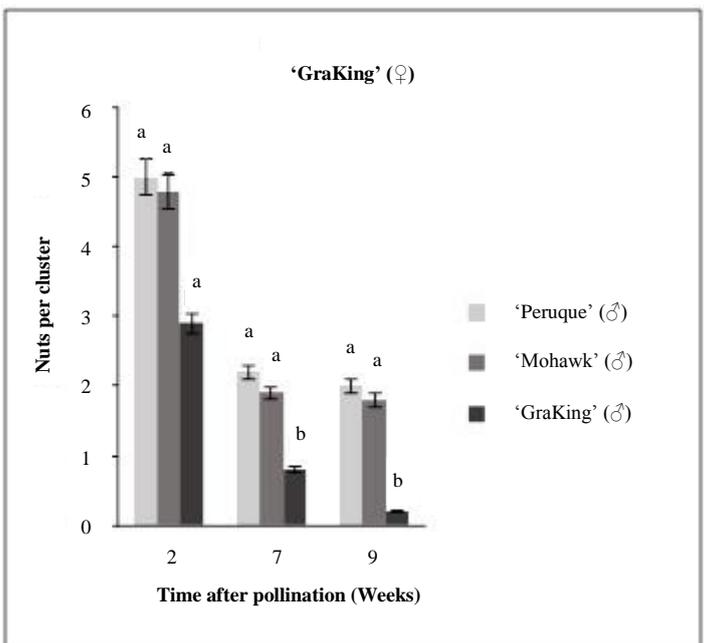
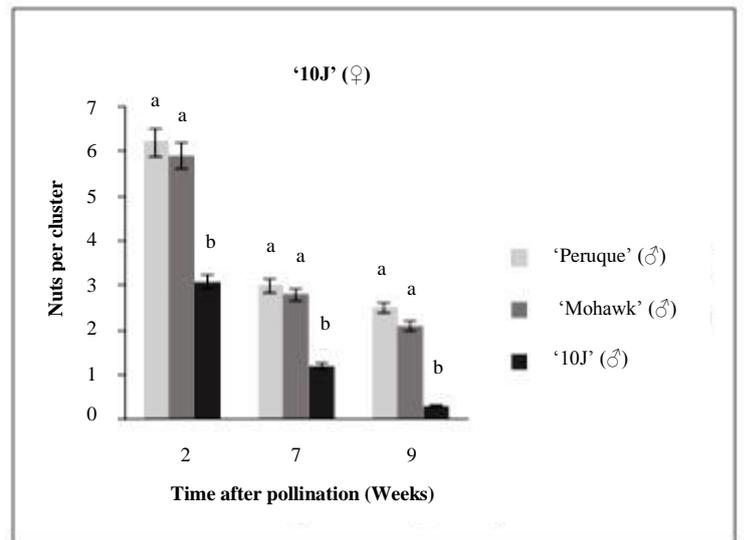
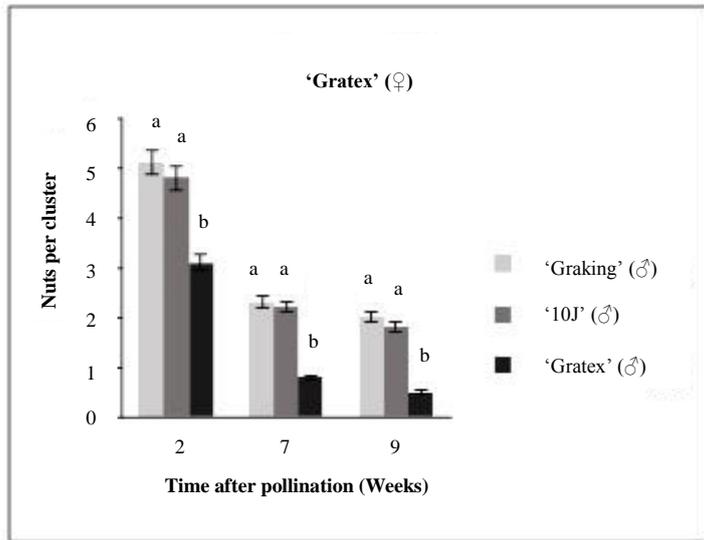


Fig. 4. Means nuts per cluster in 2, 7 and 9 weeks after pollination in five selected pecan cultivars. Means of columns at every time followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.05$).

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