

Analysis of pecan cultivars Mahan and Western in East China

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ABSTRACT. Pecan (Carva illinoensis) has been introduced to East China for over one hundred years, but its planting is still only occurring at a small scale. The key limiting factor is its low yield. To enhance the yield pecan in East China, two pecan cultivars, Mahan and Western, were examined. Twenty traits describing phasic development, yield, nut quality, and cultural practice were investigated. We found that pecan cultivar Mahan gives a higher yield and nut quality than cultivar Western. We recommend interplanting of cultivar Pawnee to act as a pollinator tree. Appropriate cultivation practices that can be implemented to enhance fruit yield of cultivars Mahan and Western include soil-applied paclobutrazol (PBZ) at certain concentrations, pinching, and supplementary pollination. For example, the addition of 1.25 g/m² of PBZ inhibits pecan branch growth and stimulates short bearing branches, which promotes fruit yield. We found that soilapplied PBZ reached optimal performance 82 days after application. A pinching length of 40 cm resulted in a fruit yield increase. In addition, grafting and transplantation may promote male flowering, but delays

female flowering. These cultural practices may provide insights that can be used to improve pecan cultivation in East China.

Key words: *Carya illinoensis*; Nut quality; Soil-applied paclobutrazol; Pinching; Supplementary pollination

INTRODUCTION

Pecan [Carya illinoensis (Wangenh.) K. Koch], also called Meiguo Shanhetao in Chinese, is a silvicultural and horticultural crop (Fowells, 1965; Flack, 1970). Pecan is native to Mexico and the Midwest and Southeastern regions of the United States. Currently, expanding from its origin, pecan has been planted over 20 countries on five continents, including Mexico, Italy, France, Israel, Japan, China, and so on (GRIN taxonomy for plant, 2015). Latitude and climate are the main factors limiting the distribution and domestication of pecan trees. In the Americas, pecan grows between 30°-33°N in latitude, with an average temperature of 15°-20°C. At the same time, temperatures below 10°C should last for longer than 750 h (Herrera, 1996). With its similar ecology, Eastern China has potential regions in which pecan may grow.

To date, many cultivars have been introduced to suitable regions of China, including pecan cultivars Western (Li et al., 2010), Mahan (Zhang et al., 2013), Tejas (Liu et al., 2013), Pawnee (Sheng et al., 2011), Shawnee (Zou et al., 2010), Shoshoni (Zhu et al., 2014), and Cardo (Zou et al., 2010). Pecan has been introduced in China for a long time and is now widely cultivated. Already in the early 19th century, missionaries and researchers brought pecan to Nanjin City, Jiangsu Province of Eastern China (Pan and You, 1994). There are approximately 200 ha of pecan grown, mainly distributed in Jiangsu Province, including the cities of Changzhou, Nanjin, and Yancheng. However, there is still no large-scale production.

Although pecan has over one hundred years' history of introduction, it is still planted only at a small scale. The key limiting factor is the low yield. To cultivate pecan at a large scale, the immediate priority is to enhance yield of pecan cultivars in Eastern China. In this experiment, two pecan cultivars Mahan and Western were used to investigate phasic development, yield, and nut quality, and cultural practices. The objective of the study was to investigate ways to enhance pecan cultivar yield in East China.

MATERIAL AND METHODS

A total of three pecan cultivars were used in these experiments. Pecan cultivars Western and Mahan were used to investigate a total of 20 traits, whereas cultivar Pawnee was used as pollinizer. All three cultivars were grown in 2007, using grafted plants. All cultivars were eight years old and grew well. The plant heights were 4.5-5.0 m. Pecan cultivar Mahan had the biggest trunk (11 cm), and cultivar Pawnee had the smallest (7 cm). These pecan trees were sparse and layered. There were 3-5 branches located in the lower layers and >4 branches in the upper layers. In addition, grafting of cultivars Western and Mahan, including one-, two-, and three-year-old grafting, was used in a transplantation experiment.

All experiments were conducted in the experimental field of Golden Land Agriculture and Animal Husbandry Science and Technology Service Company Limited (31°48'-32°03'N, 119°46'-120°01'E), between March 2012 and January 2014. This location has a north subtropical marine climate, with an annual temperature of 15.6°C, average annual precipitation of 1086

mm, and an average of 2019 sunshine hours. Hence, this location should be a suitable region to cultivate pecan.

A series of measurements were carried out, including phasic development, yield, and nut quality. The cultural practices are provided in **Table S1**. Three trees of each cultivar were selected to record dates of budding, elongation and flowering. Five trees of each cultivar were chosen to investigate pecan maturity. Subsequently, we counted theoretical yield, actual yield, average yield, over average yield and fruit based on the flowers and fruits in 2012 and 2013. We also measured the nuts size, shell thickness, nut and kernel weight, hollow kernel, crude fat and protein. Simultaneously, we also explored the effect of paclobutrazol (PBZ) soil application, pinching, supplementary pollination, grafting, and transplantation on pecan yield. Ten days after supplemental pollination, branch nuts were picked and sectioned. Likewise, 10 days after natural pollination, dropped nuts were also collected and sectioned. All data collected in these experiments were analyzed by Microsoft Office Excel v. 2014 and SPSS v. 20.00.

RESULTS

Phasic development

The pecan shoot elongation period is listed in <u>Table S2</u>. Pecan buds arose in early March. Pecan cultivars Pawnee and Mahan had budbreaks on March 10 and 26th, respectively. Pecan budding usually lasted 7-10 days. Shoots elongated from mid-March with cultivar Pawnee starting on March 14th, whereas cultivar Mahan started on April 1st. The elongation period of both cultivars lasted approximately 8-10 days.

Pecan cultivar Western started blooming on April 25th and lasted 12 days, whereas the other two cultivars did not start blooming until 6-7 days later (Table 1). Pollen was shed in cultivar Pawnee on April 30th, lasting approximately ten days. Male flowers of cultivar Western emerged on May 7th and those of cultivar Mahan on May 11th. Thus, we found that cultivar Pawnee is protandrous, whereas the other two varieties are protogynous. Pecan cultivar Western is a late pollinator of cultivar Mahan, Pawnee is a late pollinator for Western, and Pawnee can also pollinate Mahan.

Table 1	Table 1. Blooming period in three pecan (Carya illinoensis) cultivars.																				
Cultivar		April								May											
		25	26	27	28	29	30	1	2	4	5	6	7	10	11	12	13	14	15	16	17
Western	8												1	45	87	84	47	19	13	8	2
	9	13	49	82	87	95	98	99	91	63	27	15	7								
Mahan	3														1	5	22	49	62	88	97
	9								5	28	71	71	91	84	88	56	11	1			
Pawnee	3						5	23	42	47	63	85	57	29	2						
	9							7	22	38	66	81	100	59	66	74	63	52	41	30	15

The first line/column represents investigation date/cultivar, whereas the numbers in the table are flower percentage. No investigation was performed on May 8-9 and 18-19 due to had weather

Pecan nuts matured in midseason (Figure 1). Double maturing peaks appeared during the harvest time of both cultivars Western and Mahan. Nuts of the cultivar Western matured between October 12 and 29th and 60 nuts were harvested during its maturing main peak.

Pecan cultivar Mahan starting maturing on October 15th and lasted half a month. A total of 139 nuts were collected during its main maturing peak.

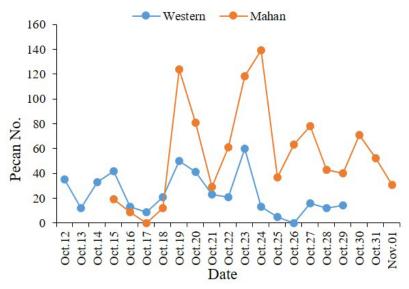


Figure 1. Nut maturation time of pecan cultivars Mahan and Western (Carya illinoensis).

Yield and nut quality

Yield traits of cultivars Western and Mahan are shown in Figure 2A. The actual yield was lower than the theoretical one. The theoretical yield of cultivar Western ranged from 207 to 439 nuts, whereas the actual yield only reached 10 to 39 nuts. The theoretical yield of cultivar Mahan ranged from 150 to 392 nuts, whereas the actual yield ranged from 73 to 201 nuts. The actual yield/theoretical yield of cultivar Mahan was approximately 50%, whereas that of cultivar Western was about 10%. The drop of cultivar Western was higher than that of cultivar Mahan. The amount of hollow kernels of cultivar Western ranged from 2 to 25%. In contrast, the amount of hollow kernels of cultivar Mahan was slightly higher. Hence, the yield traits of cultivar Mahan outperformed that of cultivar Western.

The yield stability estimates of two years of cultivar Western were similar to that of cultivar Mahan, as indicated by the proportion fruit and above average proportion fruit (Figure 2B). Compared to 2012, the fruit percentage of cultivar both Western and Mahan exhibited a small increase in 2013. In contrast, the above average fruit percentage of two cultivars presented a gentle trend in the two years. There was no significant difference of fruit percentage and above average fruit percentage between the two cultivars or across the five different planting areas.

Nut quality of cultivars Mahan and Western are shown in Table 2. The coefficient of variation (CV) of shell thickness was the highest (15.04%), whereas the CV of kernel percentage was the lowest (0.07%). Crude fat of cultivar Mahan was 61.60%, whereas that of cultivar Western was 10% higher. In terms of the other six nut quality traits, cultivar Mahan outperformed cultivar Western to a certain extent.

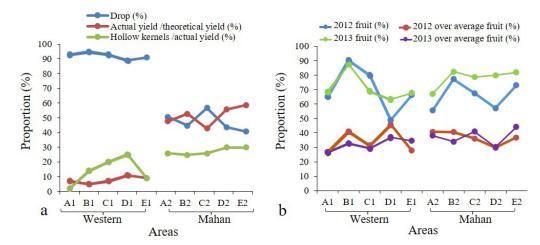


Figure 2. Yield traits and stability of cultivars Western and Mahan. **a.** Yield traits, including the drop (blue line), actual yield/theoretical yield (red line), and hollow kernels/actual yield (green line). **b.** Yield stability, including 2012 fruit (blue line), 2013 fruit (green line), 2012 over average fruit (red line) and 2013 over average fruit (purple line). A, B, C, D, and E indicate different planting areas.

Table 2. Nut quality and coefficients of variation (CV) in two different pecan (Carya illinoensis) cultivars.

Cultivar	Nut size (cm)	Nut weight (g)	Kernel weight (g)	Kernel percentage (%)	Shell thickness (mm)	Crude fat (%)	Crude protein (%)
Mahan	3.45	10.72	5.96	55.63	0.95	61.60	13.40
Western	2.93	8.76	4.87	55.57	0.93	71.90	12.47
CV (%)	11.53	14.23	14.23	0.07	15.04	10.91	5.08

Grafting and transplantation

The influence of grafting and transplantation on cultivar flowering, are shown in Table S3. Of the one-year-old cultivar Western, 3.8% produced male flowers, whereas 7.8% of one-year-old cultivar Mahan produced female flowers and 8.9% produced male flowers. In the two-year-old cultivar Western, the flower production was slightly enhanced (1.7 and 4.5% of the trees produced female and male flowers, respectively). In contrast, two-year-old cultivar Mahan produced 11.1 and 12.8% female and male flowers, respectively. In the third year, the flowering of both cultivars kept increasing. Pecan cultivar Western produced 5 and 20.5% female and male flowers, respectively. Pecan cultivar Mahan produced at least 10% more female and male flowers compared to cultivar Western. After transplantation, three-year-old cultivar Western only produced 0.56% female flowers, whereas 25% of the transplanted trees produced male flowers. Similar results were found in transplanted cultivar Mahan. Thus, it can be observed that flower maturity tends to be earlier in older cuttings and transplantation may promote male flowering, but delays female flowering.

Pinching

We found that pinching length caused differences in amount of bearing branch (Figure 3). A pinching length of 40 cm produced 71.91% bearing branch in cultivar Mahan and

72.93% in cultivar Western. When a pinching length of 50 cm was used, the bearing branch of cultivars Mahan and Western were both slightly reduced to 67.77 and 63.86%, respectively. At a pinching length of 30 cm, a further 2.10 and 1.06% bearing branch drop was observed in cultivars Mahan and Western, respectively. We found no significant treatment effects of the three different pinching lengths, but pinching significantly enhanced the amount bearing branch in the two cultivars compared with control trees (Table S4).

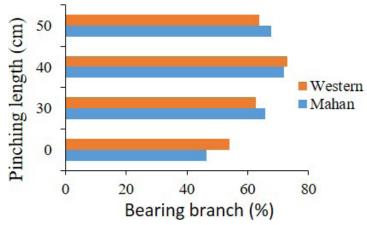


Figure 3. Effect of different pinching lengths on proportion spray in cv. Mahan (blue bars) and cv. Western (orange bars).

Effect of PBZ on response of branch growth and yield

The addition of PBZ affected both branch diameter and length as illustrated in Figure 4. The diameter of the first branch was reduced when the concentration of PBZ was $1.00~\text{g/m}^2$ for cultivar Western and $1.25~\text{g/m}^2$ for cultivar Mahan. The diameter of the second branch showed similar trends (highlighted with red dots in Figure 4A). The length of the first and second branches was significantly reduced when the concentration of PBZ was $1.25~\text{g/m}^2$ for cultivar Western and $1.50~\text{g/m}^2$ for cultivar Mahan (highlighted with red dots in Figure 4B). This suggests that cultivar Western was more sensitive to PBZ, compared to cultivar Mahan. The average growth in both branch diameter and length were reduced 82 days after the application of PBZ at different concentrations (indicated by red lines in Figures 4C and D). The trend observed in average diameter growth as a function of PBZ application was 82 < 52 < 7 < 112 < 37 < 22 days, whereas the trend observed for average length growth was 82 < 112 < 52 < 37 < 7 < 22 days. Hence, the optimal effect of PBZ is observed 82 days after application for both traits.

Soil-applied PBZ was found to effectively promote fruit yield in both cultivars Mahan and Western (Figure 5). When the PBZ concentration was 1.25 g/m², the fruit yield of cultivar Mahan reached its maximum of 98.67%. In contrast, at a PBZ concentration of 1.00 g/m², the fruit yield of cultivar Western was the highest at 92.67%. However, when the PBZ concentration was 1.25 g/m², the fruit yield of cultivar Western was 90.31%, which was only 2.36% lower than the yield of cultivar Western at 1.00 g/m² PBZ. No significant treatment effects were observed among the five different PBZ concentrations, but soil-applied PBZ

significantly enhanced the amount spray of two cultivars at 1.00-1.25 g/m² as compared with control trees (**Table S5**).

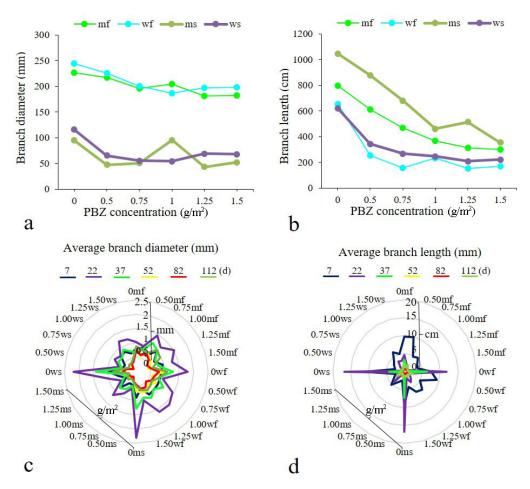


Figure 4. Effects on branch diameter and length of the addition of different concentrations of paclobutrazol (PBZ) in cultivars Mahan and Western. **a. b.** Light green and moss green lines represent cv. Mahan's first and second branch, respectively, whereas the light blue and purple lines represent the first and second branch of cv. Western, respectively. The line graphs (**a** and **b**) show the effect of PBZ concentration on total branch diameter and total branch length, whereas the radar charts (**c** and **d**) show the effect of PBZ concentration and duration of PBZ application on the average branch diameter and average branch length of the two cultivars. The differently colored lines in c and d represent duration of PBZ application (7, 22, 37, 52, 82, and 112 days). The scale bar (vertical line) in c and d indicates branch diameter (mm) and length (cm). mf: cultivar Mahan first branch, wf: cultivar Western first branch, ms: cultivar Mahan second branch, and ws: cultivar Western second branch. The PBZ concentration unit g/m² indicates the weight of PBL (g) per square meter of canopy cover.

Supplementary pollination

We found that supplementary pollination increased fruit yield in both cultivars Mahan and Western (Table 3), but no obvious influence was found on fruit set. The fruit set in both

cultivars reached at least 50%. Compared to natural pollination in cultivars Mahan and Western, the fruit yield was improved by 3.44 (cultivar Mahan) and 16.88% (cultivar Western) after supplementary pollination.

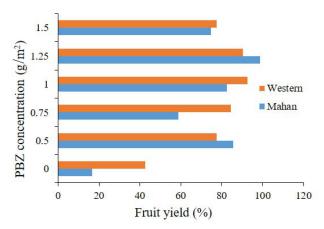


Figure 5. Fruit yield of cv. Mahan (blue bars) and cv. Western (orange bars) in relation to different concentrations of paclobutrazol (PBZ).

Table 3. Effects of supplementary and natural pollination on pecan (Carya illinoensis) fruit yield and fruit set.

Treatment	Average fruit set (%)	Average fruit yield (%)	Sum of squares	Mean square	d.f.	F value
Mahan SP	50.21	5.07	0.03	0.00	1	45.66**
Mahan NP	50.38	1.63	0.08	0.01	1	0.40
Western SP	59.63	32.24	0.50	0.05	1	53.60**
Western NP	50.07	15.36	0.14	0.01	1	6.72

SP: supplementary pollination, NP: natural pollination, ** $P \le 0.01$, highly significant difference.

Paraffin sections of two types of pecan nuts are shown in Figure 6. Ten days after supplemental pollination, branch nuts were picked and sectioned. The embryo and endosperm had formed a cystic structure (indicated by a red arrow in Figure 6A). Likewise, 10 days after natural pollination, dropped nuts were also collected and sectioned. As can be seen in Figure 6B, the ovary was solid (indicated by red arrow). The comparison between the two sections indicates that poor pollination caused nuts to drop.



Figure 6. Paraffin sections of pecan (*Carya illinoensis*) nuts. **a.** Ten days after supplementary pollination; **b.** dropped nut 10 days after natural pollination; scale bar = 1 mm. The red arrow in Figure 6A indicated a cystic structure that embryo and endosperm had formed, but the red arrow in Figure 6B indicated a solid ovary.

DISCUSSION

Although pecan has been introduced to China for more than one hundred years, it is still being planted only at a small scale. The key limiting factor is the low yield. In the following parts, we discuss potential cultural practices that may enhance pecan yield in East China.

Grafting and transplantation

Flower maturity tended to occur earlier in older cuttings. We found that transplantation promotes male flowering but delays female flowering. Pecans have mixed buds that are differentiated to first produce male flowers. Female flowers then develop following a certain time of apical growth. Older cuttings contain high nutrient stores and may flower earlier. When the grafting is transplanted, the nutrients will be redistributed. Hence, after female flowers are formed, pecan trees require more nutrition to grow and inadequate nutrition reduces male flower formation. Nutrition that may contribute to flower form was found in our study, and others, such as shoot vigor (Sparks, 1988) and environmental perturbations (Irish, 2010), also regulate both flower formation and transition, finally affecting flower production and abortion. Furthermore, high temperatures promote male flowers, but delays female flower development in cucurbit (Taylor, 1997). Temperatures of 32°C day/21°C night lead to abortion of female flower buds in pumpkins (Loy, 2004). High temperatures affect flowers is consistent with grafting and transplantation affect flowers.

Pinching

Early nut production is directly related to the amount of pinching; trees subjected to less pinching will start producing earlier than more heavily pinched trees. We found that pinching stimulated more vigorous shoot growth, which significantly increased the amount of spray compared with control trees. Crane (1933) found that pinching produced longer and stockier new shoots and observed an increase in the number pistillate flowers on weak shoots but not on strong shoots. It has been shown that pinching may increase the yield of cultivar Western (Anon, 1967). Worley (1984) also got similar result of pinching on three southeastern pecan cultivars. Of the three pinching lengths (30-50 cm) investigated in the present study, 40 cm was the optimal pinching length to promote spray in both cultivars. Observations by Xiao et al. (2014) suggested that it was most effective if the new branch was 60-80 cm.

Supplementary pollination

Pecan is monoecious dichogamous, and trees are cross-pollinated between cultivars. Self-pollination is minimal and generally does not produce high quality nuts. In this study, cultivars Pawnee, Mahan, and Western may cross-pollinate. Pecan cultivar Pawnee was a pollinator for both the other two cultivars. The same was observed by Sheng et al. (2011). Li et al. (2010) suggested that cultivar Western may also pollinate cultivar Shawnee. This is consistent with what we found in our study. Controlled or supplemental pollination is a cultural practice that has been demonstrated as an effective means of correcting a wide range of pollination-related problems. In this study, supplementary pollination may increase nut yield of cultivars Mahan and Western, but no obvious influence was found on nut set. Supplemental

pollination used to improve fruit yield is a well-recognized and established cultural practice (Dag et al., 2000; Kieran, 2000; Wells, 2007). The investigation of paraffin sections between branch and dropped nuts indicated that dropped nuts were likely caused by poor pollination in this study. Crop losses can be due to poor pollination, excessive self-pollination, or xenia (Romberg and Smith, 1946; Wood, 2000). Supplementary pollination appears to play a role in fruit drop.

PBZ effect on pecan branch and fruit

PBZ is a plant growth regulator that is widespread and effective for reducing shoot growth and increasing flower number and yield in many fruit tree species including apple (Swietlik and Miller, 1983), cherry (Quinlan and Webster, 1982), nectarine (DeJong and Doyle, 1984), pear (Ráese and Burts, 1983), and pecan (Andersen and Aldrich, 1987; Wood, 1984, 1988a,b). In this study, the effects of PBZ concentration and duration of application of soil-applied PBZ were examined. Branch growth was effectively inhibited when the PBZ concentration was 1.00-1.25 g/m² in cultivar Western and 1.25-1.50 g/m² in cultivar Mahan. A PBZ concentration of 1.25 g/m² may promote fruit yield in both cultivars. Andersen and Aldrich (1987) presented soil-applied PBZ on Cheyenne pecans at concentrations between 0.05 to 2.60 g/a.i. m² trunk cross-sectional area. Gaash and David (1989) applied 6-12 mg/a.i. cm² trunk cross-sectional area under the canopy. The concentration of soil-applied PBZ varied greatly not only between previous studies, and the PBZ concentration also had a big effect in the present study. This is likely because the soil-applied PBZ concentration may be affected by local weather, soil type, tree age, and so on. In addition, we found that soil-applied PBZ gave the best results 82 days after application. Previous studies have only reported effects of soil-applied PBZ under field conditions lasting at least 2-3 years (Andersen and Aldrich, 1987; Gaash and David, 1989). However, even a few days of PBZ application may result in optimal performance, which may be used to guide the PBZ usage of orchard workers.

In summary, we described and analyzed two pecan cultivars from East China by examining 20 yield-related traits. We found that cultivars Mahan, Western, and Pawnee may be interplanted, and appropriate cultivation practices, including soil-applied PBZ at suitable concentration ranges and suitable pinching, may promote fruit yield. We also found that transplantation may regulate flowering. However, there are some deficiencies in this study. Firstly, the experiment data was only collected from two years (2012 and 2014). More replicates would improve the experimental design and make the sampling protocols more reliable. Secondly, pest control is an important practice used in pecan cultivation, but this was not investigated in this study. Future studies will be carried out to collect more experiment data and also investigate pest control.

Conflicts of interest

The authors declare no conflict of interest.

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Supplementary material

<u>Table S1.</u> Measurements of phasic development, yield, nut quality, and cultural practices.

Table S2. Period of shoot elongation, including budding and leafing period, of three different pecan (Carya illinoensis) cultivars.

Table S3. Male and female flower number of grafted and transplanted pecan (Carya illinoensis) trees.

<u>Table S4.</u> Bearing branch effected by different pinching lengths.

Table S5. Effect on fruit yield following addition of different concentrations of paclobutrazol (PBZ).

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