November 2016. Horticultural Plant Journal, 2 (6): 301–308.

CSHS

CrossMark

Horticultural Plant Journal

Available online at www.sciencedirect.com

The journal's homepage: http://www.journals.elsevier.com/horticultural-plant-journal

A Study on the Xenia Effect in *Castanea henryi*

ZHANG Xuhui, YUAN Deyi *, ZOU Feng, FAN Xiaoming, TANG Jing, and ZHU Zhoujun

Key Laboratory of Non-wood Forest Nurturing and Protection of the National Ministry of Education, Central South University of Forestry and Technology, Changsha 410004, China

Received 5 May 2016; Received in revised form 8 October 2016; Accepted 3 November 2016

Available online 15 February 2017

Abstract

The xenia effect results in pollen affecting the setting rate and the appearance and quality of fruits. To further understand this phenomenon, we investigated the xenia effect in *Castanea henryi* using the cultivars 'Huali 1', 'Huali 2', 'Huali 3', and 'Huangzhen' as materials. Twenty combinations of self-, cross-, and natural pollination were undertaken in a chestnut orchard in Chenzhou City, Hunan Province, China. Significant differences were observed among the pollination combinations in terms of the time of fruit ripening, rate of fruit setting, size of the barbed shell and nut, and content of soluble sugars, fats, proteins, amylose, and vitamin C. No significant differences were observed with regard to nut rate, content of total starch, and moisture content. The fruit quality of the 20 pollination combinations was evaluated using a subordinate function method, and showed that Huali 2 × Huali 3 was the best combination, and Huangzhen × Huangzhen was the most ineffective combination. Therefore, we recommend the combination of Huali 2 × Huali 3 for the future production of *C. henryi*.

Keywords: *Castanea henryi*; fruit-setting rate; pollination; subordinate function method; xenia

1. Introduction

The xenia effect is the direct influence that pollen has on the characteristics of seeds and fruits in the fertilized plant (Denny, 1992). The xenia effect in cross-pollinated plants not only influences the fruit set, but also determines the shape, maturity, size, color, flavor, and composition of fruits (Alexander et al., 2012). In recent years, research into the xenia effect has focused on improving the quality of crops of almond, apple, litchi, grape, chestnut, pear, kiwifruit, momordica grosvenori, pecan, etc. (Kumar and Das, 1996; Qiu et al., 2006; Sha et al., 2006; Wang et al., 2010, 2012). However, to our knowledge, there have been no such studies on the chestnut (*Castanea henryi* Rehd. et Wils.). In the present study, four varieties of *C. henryi* were used as test subjects. The xenia effect brought about by the pollen of these varieties was identified in different experimental groups using different combinations of self- and out-crossing pollination field experiments. The results of this study provide a theoretical basis for high-efficiency cultivation of *C. henryi*.

2. Materials and methods

2.1. Materials

The study was conducted for two years (2013–2014) in the south chestnut experimental site at the Central South University of Forestry and Technology in Rucheng County, Chenzhou City, Hunan Province, China (25°33′43″N, 113°45′08″E). The study site is located at an altitude of 765 m above sea level and situated in a subtropical monsoon climate. The annual rainfall is 1 547.1 mm with an average annual temperature of 16.6 °C.

The test specimens were 4 separate cultivars of *C. henryi* (Rehd. et Wils.): Huali 1 (H1), Huali 2 (H2), Huali 3 (H3), and Huangzhen (H). We conducted 20 combinations of self-,

* Corresponding author. Tel.: +86 15111264681

E-mail address: yuandeyi@126.com

Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

The Chinese version of this paper is published in Acta Horticulturae Sinica. DOI: 10.16420/j.issn.0513-353x.2015-0384

http://dx.doi.org/10.1016/j.hpj.2017.02.003

cross-, and natural pollination using these 4 varieties. Eight-year-old healthy, moderate-sized trees were selected for the experiments.

2.2. Experimental methods

A total of 180 trees were selected for the experiment, and each variety was selected with a total of 45 plants, each randomized design was composed of 3 plants with three replications. Firstly, vegetative shoots above, within, and below the tree crown were emasculated and bagged. Fifty shoots were bagged per plant, each labeled individually. Secondly, when the male flowers bloomed, pollen from every variety of *C. henryi* was collected and stored, respectively. Thirdly, when female stigmas opened at an angle between 30° and 45°, the plant was pollinated, bagged, and properly labeled. We used naturally pollinated flowers as the control.

2.3. Determination and analyses

The characteristics of fruit ripening from different pollination combinations were measured from late August to September in 2013 and 2014. The experiment began when the percentage of blooming buds reached 30% and concluded when it reached 90%. Samples were collected and the bud setting rate, fruition rate, and seeding rate were calculated for each combination. The equations used were as follows: bud setting rate (%) = (bud number/pollination number) × 100; fruition rate (%) = (seed number/pollination number) × 100; seeding rate (%) = (average weight per nut/average weight per bud) × 100; empty bud rate = bud setting rate − fruition rate.

The weight of the fruit was determined by averaging the weights of all the collected buds and the nuts inside each bud. The width of buds and nuts was directly measured with a Vernier caliper and averaged. The length of buds and nuts was directly measured from the base to the tip using a Vernier caliper.

Seeds were dried at 70 °C, crushed, and stored. Detection of soluble sugar content was conducted using anthrone colorimetry (Chen, 2010); amylose content was determined using the dual-wavelength method (Chen et al., 2012); total starch content was measured using the anthrone colorimetric method (Liang et al., 2009); crude fat content was determined by the Soxhlet extraction method (Wang et al., 2008); protein content was measured using the Kjeldahl method (Bao, 2005); and vitamin C content was measured using the dichlorophenolindophenol colorimetric method (Tong and Zhou, 1982).

All presented data are the averages of each cell. Analysis of variance was performed using SPSS 19.0.

3. Results and analyses

3.1. Xenia effects on fruit ripening

We observed specific effects on the maturity of fruits when different varieties of *C. henryi* were crossed with different paternal pollens. After natural pollination, Huali 1 was an early maturing variety, Huali 2 and Huangzhen were intermediate maturing varieties, and Huali 3 was a late maturing variety. Under controlled pollination, paternal pollens from the four different sources had significant impacts on the maturity of the maternal fruit. As shown in Table 1, fruits from Huali 1 pollinated by Huali 2, and fruits from Huali 2 and Huali 3 pollinated by Huali 1, had accelerated growth and reached maturity 2–3 d earlier than natural pollination. In contrast, fruits from Huali 1 pollinated by Huali 3, fruits from Huali 2 pollinated by Huangzhen, and fruits from Huali 3 pollinated by Huali 2 had delayed growth and took 4–5 d long to reach maturity. These results were consistent with the growth patterns of the pollen donors. However, regarding cross-pollinations of Huangzhen, pollen from Huali 3 resulted in early maturity, whereas pollen from Huali 1

Table 1 Xenia effects on bur cracking time of *C. henryi* fruits

♀	♂	August				September																									
		28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
H1	CK			★	★	★	★	★	★																						
	H1			★	★	★	★	★																							
	H2	★	★	★	★	★	★																								
	H3							★	★	★	★	★	★																		
	H				★	★	★	★	★	★																					
H2	CK														★	★	★	★	★	★	★	★									
	H2															★	★	★	★	★	★										
	H1												★	★	★	★	★	★	★												
	H3															★	★	★	★	★	★										
	H																		★	★	★	★	★	★							
H3	CK																						★	★	★	★	★	★			
	H3																														
	H1																			★	★	★	★	★	★	★					
	H2																								★	★	★	★	★	★	★
	H																							★	★	★	★	★			
H	CK																		★	★	★	★	★	★	★						
	H																			★	★	★	★	★	★						
	H1																				★	★	★	★	★	★	★				
	H2																			★	★	★	★	★	★						
	H3														★	★	★	★	★	★	★	★									

Note: H1: Huali 1; H2: Huali 2; H3: Huali 3; H: Huangzhen; CK: Natural pollination.

resulted in the most delayed maturity, which was a result inconsistent with the growth patterns of the pollen donors. Therefore, the fruit ripening period for different pollination combinations usually, but not always, followed the characteristics of the pollen donor.

3.2. *Xenia effects on fruition*

As shown in Fig. 1, cross-pollination increased female bud setting rate compared to natural pollination and self-pollination. Specifically, the bud setting rate was the highest with Huangzhen as the recipient. When Huali 3 was the recipient, the increase in bud setting rate was not significant compared with other cross-pollination combinations. Among other combinations, pollens from Huali 2 and Huangzhen significantly increased the bud setting rate of Huali 1, whereas pollen from Huali 3 significantly increased the bud setting rate of Huali 2. Compared with self-pollination, cross-pollination combinations with Huali 2 and Huangzhen as recipients resulted in the most significant increase in bud setting rate. Among all pollination combinations, Huali 1 pollinated by Huangzhen had the highest bud setting rate (83.33%), and Huali 2 pollinated by Huali 3 had the highest increase in bud setting rate compared to self-pollination (64.21%).

Crosses using different paternal pollens also had substantial effects on the fruition rates of maternal plants (Fig. 1). Compared to self-pollination, every cross-pollination combination resulted in a significant increase in fruition rate. Specifically, Huangzhen pollinated by Huali 3 had the highest fruition rate (80%). Compared to natural pollination, cross-pollination also resulted in increases in the fruition rate, with the exception of the (H2 × H1) combination. Specifically, combinations with Huangzhen as the recipient had the highest increase in fruition rate. Among other combinations, pollen from Huali 2 significantly increased the fruition rate of Huali 1, and pollen from Huali 3 significantly increased the fruition rate of Huali 2. The trend between the bud setting rate and fruition rate of cross-pollinated *C. henryi* was uniform.

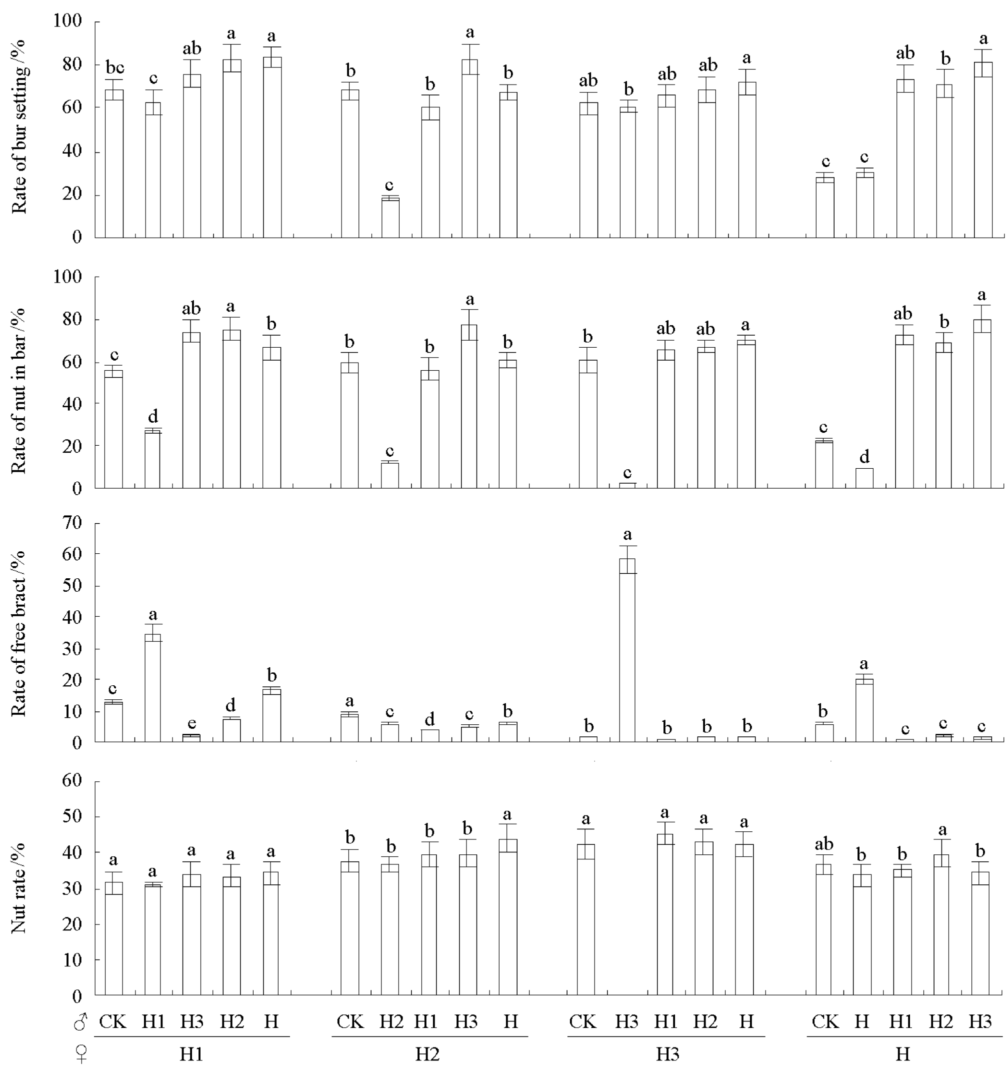

Fig. 1 Rate of bur setting, nut in bar, free bract and nut rate of different combinations
H1: Huali 1; H2: Huali 2; H3: Huali 3; H: Huangzhen; CK: Natural pollination.

Table 2 Effect of different pollen sources on phenotypic character of fruit

Female	Male	Nut size/g	Longitudinal diameter/mm	Transverse diameter/mm
H1	CK	10.25 ± 0.93 b	29.11 ± 1.69 c	25.06 ± 0.73 b
	H2	13.19 ± 1.17 a	32.68 ± 1.27 a	27.09 ± 1.13 a
	H3	10.49 ± 0.86 b	30.68 ± 1.61 b	24.95 ± 1.70 b
	H	12.52 ± 0.59 a	31.68 ± 1.36 ab	25.56 ± 1.19 b
H2	CK	7.23 ± 0.73 c	23.11 ± 1.27 b	21.75 ± 0.96 c
	H1	9.26 ± 0.83 a	25.03 ± 0.97 a	23.68 ± 1.30 a
	H3	8.67 ± 0.69 ab	25.50 ± 1.34 a	23.38 ± 1.55 ab
	H	7.64 ± 0.92 b	23.33 ± 1.65 b	22.30 ± 1.40 bc
H3	CK	8.37 ± 0.79 ab	24.65 ± 1.49 c	22.72 ± 1.75 ab
	H1	9.91 ± 0.97 a	26.47 ± 1.05 a	23.95 ± 0.83 a
	H2	7.99 ± 0.29 b	25.11 ± 1.58 bc	22.85 ± 1.56 ab
	H	9.64 ± 0.81 ab	26.24 ± 1.28 ab	22.36 ± 1.84 b
H	CK	10.21 ± 1.06 b	27.39 ± 2.38 b	26.26 ± 1.76 ab
	H1	13.53 ± 1.01 a	30.52 ± 1.65 a	27.41 ± 1.20 a
	H2	10.02 ± 0.71 b	27.86 ± 1.33 b	24.25 ± 1.39 c
	H3	11.20 ± 0.86 b	28.30 ± 1.57 b	25.75 ± 1.89 b

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

The self-pollination fruition rate for all varieties of *C. henryi* was generally low. For the four test varieties, the self-pollination fruition rates were all lower than 30.00%, with the rate of Huali 3 < Huangzhen < Huali 2 < Huali 1. Specifically, the self-pollination fruition rates of Huali 1, Huali 2, Huali 3, and Huangzhen were 27.50%, 12.43%, 2.00%, and 10.00%, respectively. Fruition rate was inversely related to the empty bud rate; i.e. the higher the fruition rate, the lower the empty bud rate. Seeding rate for cross-pollination combinations was generally higher than for natural pollination, with the exception of maternal Huangzhen plants, but the difference was not significant.

3.3. *Xenia effects on physical attributes of fruit*

Pollens from different paternal varieties had obvious effects on the physical attributes of *C. henryi* fruit. As shown in Table 2, natural pollination resulted in the following rankings for fruit size: Huali 1 > Huangzhen > Huali 3 > Huali 2. Following cross-pollination experiments, the pollens from Huali 1 and Huangzhen increased the mass of fruits. Specifically, pollen from Huali 1 had the greatest effect when pollinating Huangzhen, resulting in a unit mass of 13.53 g, an increase of 3.32 g relative to natural pollination for this variety. Huali 3 and Huangzhen pollinated by Huali 2 had reduced fruit mass relative to natural pollination, which was consistent with the characteristics of the pollen donor. However, Huali 1 pollinated by Huali 2 had an increase in fruit mass, contrary to the characteristics of the pollen donor. The longitudinal and transverse diameters of the fruit also increased when mass increased.

Different paternal pollens had specific effects on the color of *C. henryi* fruits. The 'Description, specifications and standards of chestnut germplasm resources' (Liu, 2006) describes each variety as follows: Huali 1 and Huali 3 are reddish brown, Huali 2 is purplish brown, and Huangzhen is yellowish brown. The color intensity is ranked as Huali 2 > Huali 1 = Huali 3 > Huangzhen. As shown in Fig. 2, Huali 1 and Huali 2 pollinated by Huangzhen resulted in lighter skinned fruits compared to fruits resulting from natural pollination for these two varieties, whereas Huangzhen pollinated by Huali 2 and Huali 3 resulted in a darker skinned fruit. These results were consistent with the color of the pollen donors. In contrast, Huali 3 pollinated by Huali 2 resulted in a lighter skinned fruit than the skin color of the pollen donor.

These results demonstrate the role of the xenia effect in determining longitudinal and transverse diameter, fruit mass, and skin color of *C. henryi* nuts.

3.4. *Xenia effects on fruit quality*

3.4.1. *Kernel moisture content*

Different paternal pollens had a minimal impact on the moisture content of *C. henryi* seeds (Table 3). Among all pollination combinations, only crossing Huali 2 with pollen from Huangzhen

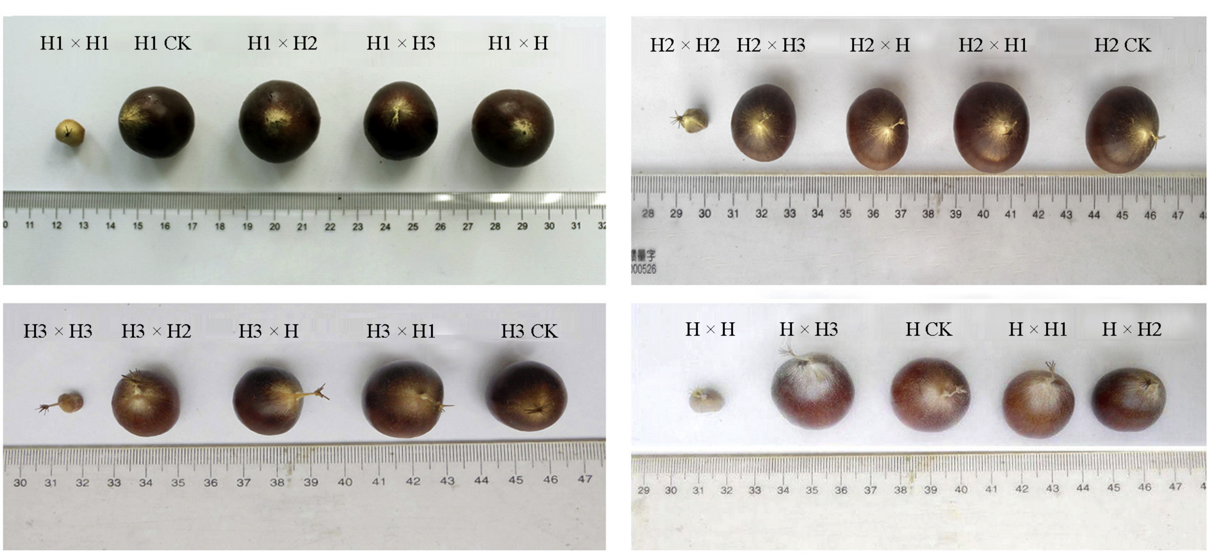

Fig. 2 Comparisons of fruit sizes and colors for different pollination combinations
H1: Huali 1; H2: Huali 2; H3: Huali 3; H: Huangzhen; CK: Natural pollination.

Table 3 Xenia effects on moisture content of *C. henryi* seeds %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	45.40 a	47.05 a	48.03 a	47.24 a	48.64 a	47.27	6.66
H2	44.46 ab	42.87 b	43.61 b	48.70 a	43.44 b	44.62	11.97
H3	46.23 a	46.93 a	–	47.61 a	46.00 a	46.69	3.38
H	50.34 ab	51.53 a	48.11 ab	46.23 b	46.93 ab	48.63	11.29

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

resulted in a significant increase in the moisture content of Huali 2 relative to natural pollination. These results suggest that the xenia effect does not play an important role in determining the moisture content of seeds for the four varieties.

3.4.2. Soluble sugar content

Different paternal pollens had significant effects on the soluble sugar content of *C. henryi* fruits (Table 4). Cross-pollination combinations with Huali 1 as the maternal recipient had the highest average soluble sugar content (7.87%). The average soluble sugar content values for Huali 2, Huali 3, and Huangzhen were 7.47%, 7.85%, and 5.91%, respectively, significantly affected by the maternal parent. The degree of change in soluble sugar content was also different for each variety: Huali 1 = 35.08%, Huali 2 = 20.40%, Huali 3 = 26.91%, and Huangzhen = 21.87%. Xenia effects between different varieties were significantly different, with the highest and the lowest values of 10.49% and 5.36%, respectively, and a degree of change up to 48.90%.

3.4.3. Crude fat content

Different paternal pollens had significant effects on the fat content of *C. henryi* fruits (Table 5). Cross-pollination combinations with Huali 2 as the maternal recipient all resulted in significantly higher values of crude fat content, and also the largest overall increase of fat content (27.55%). The maximum and minimum values were 2.94% and 2.13%, respectively. The differences between the average values from cross-pollination with different recipients were significant. Combinations with Huali 2 as the maternal recipient had an average value of only 2.64%, whereas those with Huangzhen as recipient had an average of 4.60%. As Huali 2 had an overall change of 27.55%, this suggests that the xenia effect can dramatically influence fat content. However, the case of Huangzhen only having an increase of 7.79% suggests the xenia effect may not always have a strong influence on the fat content of *C. henryi* fruits. These results suggested that the fat content of *C. henryi* exhibited xenia effects but large variations existed between different varieties.

Table 4 Xenia effects on soluble sugar content of *C. henryi* fruits %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	6.81 ± 0.34 c	7.00 ± 0.42 c	10.49 ± 0.32 a	7.79 ± 0.61 b	7.26 ± 0.41 bc	7.87	35.08
H2	7.85 ± 0.44 ab	7.05 ± 0.30 c	7.32 ± 0.31bc	8.43 ± 0.47 a	6.71 ± 0.52 c	7.47	20.40
H3	7.78 ± 0.25 b	8.77 ± 0.65 a	–	8.44 ± 0.15 ab	6.41 ± 0.36 c	7.85	26.91
H	5.59 ± 0.35 bc	5.36 ± 0.19 c	6.86 ± 0.52 a	5.42 ± 0.26 c	6.30 ± 0.44 ab	5.91	21.87

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

Table 5 Xenia effects on crude fat content of *C. henryi* fruits %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	3.20 ± 0.18 b	3.38 ± 0.16 ab	3.63 ± 0.08 a	3.44 ± 0.11 ab	3.30 ± 0.18 ab	3.39	11.85
H2	2.13 ± 0.06 c	2.88 ± 0.12 ab	2.56 ± 0.14 b	2.94 ± 0.13 a	2.69 ± 0.11 a	2.64	27.55
H3	3.44 ± 0.08 a	3.25 ± 0.16 ab	–	2.81 ± 0.13 b	3.38 ± 0.15 a	3.22	18.31
H	4.63 ± 0.06 a	4.38 ± 0.09 a	4.66 ± 0.14 a	4.56 ± 0.15 a	4.75 ± 0.12 a	4.60	7.79

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

Table 6 Xenia effects on protein content of *C. henryi* fruits %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	3.30 ± 0.27 b	3.67 ± 0.14 a	2.67 ± 0.22 d	3.00 ± 0.19 c	3.67 ± 0.12 a	3.262	27.25
H2	3.31 ± 0.11 a	2.35 ± 0.24 c	2.61 ± 0.18 b	2.39 ± 0.19 c	2.67 ± 0.23 b	2.664	28.79
H3	3.32 ± 0.24 b	2.98 ± 0.09 c	–	3.17 ± 0.20 bc	3.67 ± 0.15 a	3.290	18.80
H	2.33 ± 0.17 a	2.04 ± 0.20 b	1.96 ± 0.11 b	1.86 ± 0.17 b	2.05 ± 0.18 b	2.048	20.17

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

Table 7 Xenia effects on starch content of *C. henryi* fruits %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	61.71 ± 3.43 a	63.57 ± 5.35 a	65.98 ± 4.54 a	66.13 ± 2.15 a	56.69 ± 3.43 a	62.82	14.27
H2	61.24 ± 2.08 a	64.77 ± 4.42 a	65.01 ± 3.88 a	68.38 ± 5.16 a	67.67 ± 3.92 a	65.41	10.44
H3	62.37 ± 5.39 a	62.09 ± 0.92 a	–	64.56 ± 1.3 a	63.33 ± 4.28 a	63.09	3.83
H	63.68 ± 2.13 a	66.34 ± 5.70 a	63.51 ± 4.02 a	64.28 ± 3.09 a	63.15 ± 4.01 a	64.19	4.81

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

3.4.4. Protein content

Different paternal pollens had effects on the protein content of *C. henryi* (Table 6). Huali 2 had the greatest overall increase in protein content (28.79%). Among all the combinations, the maximum value was 3.67%, whereas the minimum was only 1.86% with a degree of change of 49.32%. Huali 1 × Huali 3 and Huali 3 × Huali 2 had significantly reduced protein content relative to the self- and naturally pollinated plants of Huali 1 and Huali 3. These results indicate that the protein content of *C. henryi* exhibited xenia effects.

3.4.5. Starch content

Amylose content can serve as an indicator for evaluating the waxy quality of chestnuts (Liang et al., 2009). Different paternal pollens had no significant effects on the total starch content (Table 7) but had an effect on the amylase content of *C. henryi* fruits (Table 8). The amylose content significantly increased with combinations of Huali 2, Huali 3, and Huangzhen as maternal recipients. Out of the four varieties, Huangzhen had the highest increase at 29.05%, with the maximum and minimum values of 15.73% and 11.16%, respectively. The maximum and minimum values for all combinations were 17.45% and 11.16%, respectively, with a degree of change of 36.05%, slightly lower than soluble sugar. Therefore, the xenia effect was demonstrated through changes in the amylose content of *C. henryi* fruits brought about by different paternal pollens.

3.4.6. Vitamin C content

Different paternal pollens had significant effects on the vitamin C content of *C. henryi* fruits (Table 9). Among the four varieties, Huali 2 as the maternal recipient had the highest average vitamin C content of 298.11 mg·kg^{-1}. Among all the combinations, Huali 2 × Huali 1 had the highest vitamin C content at 327.75 mg·kg^{-1}. The overall relative increases of cross-pollination over self-pollination and natural pollination were 40.65 mg·kg^{-1} and 25.19 mg·kg^{-1}, respectively. Combinations with Huali 3 as the maternal recipient had significantly increased vitamin C content when pollinated by the other three varieties, and also had the highest degree of change at 21.09%. Together, these results demonstrate that the vitamin C content of *C. henryi* fruits was influenced by the xenia effect.

3.5. Comprehensive evaluation of yield and fruit quality using a subordinate function method

Based on previous studies (Chen et al., 2000; Zheng et al., 2003; Guo et al., 2013) and using the fruit characteristics of *C. henryi* measured here and their economic impacts, the yield and the fruit quality were given weighted factors of 0.6 and 0.4, respectively. Under yield, the bud setting rate, nut rate, and fruition rate were given weighted factors of 0.1, 0.1, and 0.4, respectively. Under fruit quality, the content of soluble sugar, amylose, fat, protein, vitamin C, and the quality of nuts were given weighted factors of 0.13, 0.09, 0.04, 0.04, 0.04, and 0.06, respectively.

Table 8 Xenia effects on amylase content of *C. henryi* fruits %

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	12.60 ± 0.52 b	13.17 ± 0.49 ac	12.36 ± 0.60 b	12.35 ± 0.87 b	14.10 ± 1.05 a	12.92	12.41
H2	12.70 ± 1.25 b	16.32 ± 0.66 a	16.40 ± 1.05 a	17.45 ± 1.24 a	15.44 ± 0.61 a	15.66	27.22
H3	16.76 ± 1.30 a	15.50 ± 0.42 a	–	15.33 ± 0.51 a	12.98 ± 0.85 b	15.14	22.73
H	11.16 ± 0.91 c	15.73 ± 1.45 a	13.71 ± 0.82 b	13.58 ± 0.83 ab	15.00 ± 0.86 ab	13.84	29.05

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

Table 9 Xenia effect on vitamin C content of *C. henryi* fruits mg·kg^{-1}

Female	Male					Average value	Variance range
	H1	H2	H3	H	CK		
H1	280.58 ± 5.49 b	304.16 ± 0.65 a	285.46 ± 4.58ab	266.54 ± 9.77 b	301.25 ± 6.97a	287.60	12.37
H2	327.75 ± 2.03 a	287.10 ± 3.48bc	293.55 ± 0.91bc	279.58 ± 13.40 c	302.56 ± 9.26b	298.11	14.7
H3	292.12 ± 3.87 b	230.59 ± 3.16 b	–	242.63 ± 1.37 a	230.50 ± 1.07b	248.96	21.09
H	250.38 ± 3.67ab	230.37 ± 4.01 b	240.05 ± 5.52ab	253.41 ± 5.85 ab	263.90 ± 1.67a	247.62	12.71

Note: Different lowercase letters indicate significant differences at $P \leq 0.05$ level.

Table 10 Comprehensive score and ranking of each pollination combination evaluated by subordinate function method

Male	Female	Comprehensive score	Ranking	Male	Female	Comprehensive score	Ranking
H1	CK	0.541	15	H2	CK	0.589	11
	H1	0.325	16		H2	0.247	18
	H2	0.667	8		H1	0.575	12
	H3	0.718	2		H3	0.727	1
	H	0.618	9		H	0.675	7
H3	CK	0.557	14	H	CK	0.305	17
	H3	–	–		H	0.162	19
	H1	0.714	3		H1	0.563	13
	H2	0.686	6		H2	0.613	10
	H	0.688	5		H3	0.689	4

A value was given to each fruit characteristic from each category, with the higher the value the better the quality of fruit. Thus, a semi-trapezoidal-shaped distribution membership function (Guo et al., 2013) was adopted to evaluate the data.

As shown in Table 10, based on the comprehensive evaluation of yield and fruit quality, Huali 2 × Huali 3 was considered the best combination with a comprehensive score of 0.727.

4. Discussion

In this study, we found substantial evidence of the xenia effect operating in the commercially important crop plant, *C. henryi*. Fruit characteristics relating to fruit yield (fruit ripening, setting rate, fruition rate) and fruit quality (soluble sugar, fat, protein, amylose, and vitamin C content) all demonstrated the potential to be positively influenced by the xenia effect, depending on the source of the parent pollen. However, some characteristics of fruit yield and quality were not subject to the xenia effect, such as nut rate, total starch, and moisture content.

The xenia effect was most obvious in maturation time of *C. henryi*, such as the case of Huali 2 and Huali 3 pollinated by the early-maturing variety Huali 1, which accelerated fruit maturation times for the former two varieties. Self-pollinated Huali 3 only had a fruition rate of 2%, whereas other self-pollinated varieties also had low fruition rates, suggesting that *C. henryi* is self-incompatible. In addition, when compared to self-crossing the out-crossing pollination experiments had slightly improved fruition rates, which is consistent with the findings of Fan et al. (2014). These results demonstrate that the selection of suitable pollen will be an advantage when aiming to control the specific production needs of *C. henryi.*

The results revealed that different varieties of *C. henryi* were subject to varying levels of the xenia effect (i.e. different fruit attributes were affected at different intensities). For example, combinations with Huali 2 as the maternal recipient of pollen exhibited xenia effects in their fruit's soluble sugar, fat, protein, amylose, and vitamin C content, with the differences being statistically significant. Contrastingly, combinations with Huali 1 as the maternal recipient of pollen only had significant increases in their fruit's soluble sugar, protein, and vitamin C content. In addition, when pollinating different maternal recipients, the paternal pollen can cause the same beneficial xenia effects in multiple progeny plants. For example, Huali 1, Huali 2, and Huali 3 pollinated by Huangzhen had significantly increased bud setting rates compared with their self-pollinated conspecifics. The fruit quality of *C. henryi* was also subject to the xenia effect. For example, the fat content of Huali 2 significantly increased when pollinated by Huali 3 relative to its self-pollinated conspecific, whereas the same pollen donor (Huali 3) had no effect on Huangzhen and significantly reduced the fat content in Huali 1. These findings were consistent with the study published by Ma et al. (2008) and Qi et al. (2007). Furthermore, when evaluating the vitamin C content in *C. henryi*, it was relatively high, nearly three times of an apple (Lu et al., 2003).

The analyses using the average membership function showed that a pollination combination of Huali 2 × Huali 3 had the highest yield and the best fruit quality. It was also found that combinations with pollen from Huali 3 resulted in increased weighted scores. Together, our observations demonstrate that an understanding of the xenia effect in specific crop plants offers substantial benefits to the agricultural and horticultural industry. In particular, here we have identified several useful paternal and maternal combinations that take advantage of the xenia effect that can be used to enhance the production of the chestnut *C. henryi* in the future.

Acknowledgment

Financial support was provided by the '12th Five-Year' Science and Technology Support Program of China (2013BAD14B04).

References

Alexander, C.M., Stuart, A.C., Maria, A.B., 2012. Effects of natural and artificial pollination on fruit and offspring quality. Basic Appl Ecol, 13: 524–532.

Bao, S.D., 2005. Soil and Agricultural Chemistry Analysis. China Agriculture Press, Beijing.

Chen, J.J., 2010. Effects of Chinese chestnut (*Castanea mollissima* B L.) metaxenia on fruit quality [M. D. Dissertation]. Southwest Forestry University, Yunnan. (in Chinese)

Chen, X., Tao, Z.L., Wu, Z.X., 2012. Effect of PP_{333} and ethrel treatment on endogenous hormones and carbon and nitrogen nutrients in Feizixiao litchi. Acta Agric Univ Jiangxiensis, 34: 27–33. (in Chinese)

Chen, Z.X., Lei, Z.X., Liu, H.N., Wu, G.Y., Wang, X.B., 2000. Analysis of chestnut kernel nutrient ingredients and its integrated quality evaluation. J Fruit Sci, 17: 286–289. (in Chinese)

Denny, J.O., 1992. Xenia includes metaxenia. Hortscience, 27: 722–728.

Fan, X.M., Yuan, D.Y., Tang, J., Tian, X.M., Zhang, X.H., Wang, B.F., Tan, X.F., 2014. Biological characteristics of flowering and pollination in *Castanea henryi*. Sci Silvae Sin, 50: 42–48. (in Chinese)

Guo, S.J., Lv, W.J., Zou, F., Xie, P., 2013. Comprehensive evaluation and screening of different pollination combinations of chestnut based on different evaluation methods. J Beijing For Univ, 35: 42–47. (in Chinese)

Kumar, K., Das, B., 1996. Studies on xenia in almond. J Hortic Sci, 71: 545–549.

Liang, L.S., Xu, J., Wang, G.X., Ma, H.L., 2009. Relationship between starch pasting amylose content and starch granule size in different Chinese chestnut variety groups. Sci Agric Sin, 42: 251–260. (in Chinese)

Liu, Q.Z., 2006. Descriptors and Data Standard for Chestnut. China Agriculture Press, Beijing.

Lu, Y.M., Gao, H., Zhao, Z.Y., Wang, L.C., Yuan, J.J., 2003. Analysis and evaluation on fruit quality on quality of Pink Lady apple. J Fruit Sci, 20: 503–505. (in Chinese)

Ma, X.J., Shi, L., Mo, C.M., 2008. Xenia effect on main qualitative characters of *Siraitia grosvenorii*. Acta Hortic Sin, 35: 1695–1700.

Qi, X.J., Han, L.X., Li, M., 2007. Studies on pollen xenia of kiwifruit. J Fruit Sci, 24: 774–777. (in Chinese)

Qiu, Y.P., Dai, H.F., Li, Z.Q., 2006. Effects of pollinator on fruit quality of Guiwei litchi cultivar. J Fruit Sci, 23: 703–706. (in Chinese)

Sha, H.F., Zhu, Y.D., Gao, Q.J., 2006. Effect of xenia on fruit quality of Jingbaili pear cultivar. J Fruit Sci, 23: 287–289. (in Chinese)

Tong, A.Y., Zhou, H.J., 1982. Nutrition Diagnosis of Fruit Trees. China Agriculture Press, Beijing.

Wang, G.P., Liu, Q.X., Kong, D.J., 2008. Determination of amylopectin in *Castanea mollissima* BL. by dual-wavelength spectrophotometry. J Hebei Agric Sci, 12: 53–56. (in Chinese)

Wang, Q., Su, S.C., Zhao, D., Kou, Y.R., 2012. Study on varieties combination suitable for mutual pollination of Yan Mountain Chestnut. Nat Resour, 3: 66–70.

Wang, Z.J., Zhang, B., Xia, G.H., 2010. Analysis of the progeny of *Carya cathayensis* × *C. illinoensis* and the xenia effect. J Fruit Sci, 27: 908–913. (in Chinese)

Zheng, C.L., Wu, S.H., Yu, W.Q., 2003. Analyses of nutrient ingredients and quantity evaluation in nuts of Chinquapin (*Castanea henryi*). J Fujian Coll For, 23: 293–296. (in Chinese)