

Research paper

## Influences of Different Pollen Sources on Fruit Quality and Productivity of Chestnut (*Castanea mollissima* Blume) in China

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### [ Summary ]

The chestnut (*Castanea mollissima* Blume) has a very large cultivation area and production in the world and is an important nut species crop worldwide. It is also an important food and timber crop in many parts of China. It is known that pollination plays an important role in the fruit quality and fruit productivity of this species. To investigate its role, 8 locally selected pollen sources were tested for their effect on indicators of fruit quality and fruit productivity of ‘Yanshanzaofeng’ (YSZF) trees. Natural pollination served as the control experiment. Chestnut has the characteristic of alternate year bearing, so data of 2 yr was averaged and used as the final data in the study on chestnut productivity. Cross pollination of these recipient trees by ‘Zipo’ (ZP) pollen led to the highest fruit set at 86.67%, ‘Dabanhong’ (DBH) pollen led to the highest total yield (1.62 kg tree<sup>-1</sup>), ‘Qianxizhuangli’ (QXZL) pollen resulted in the highest kernel percentage (54.71%), whereas self-pollination resulted in the lowest kernel percentage. Self-pollination led to a heavier single-grain weight than did the other treatments. ZP pollen resulted in the lowest fat and the highest crude protein contents. ‘Qianxizaohong’ (QXZH) pollen resulted in the highest percentage of soluble sugars. ‘Yanshanduanzhi’ (YSDZ) pollen resulted in the highest amylopectin content, and DBH pollen resulted in the highest amylose content. The pollen of DBH and ‘Yanlong’ (YL) ranked best in a comprehensive analysis of all data using a gray relational analysis (GRA), with self-pollination ranking last and natural pollination ranking next to last. Thus, in this study, pollen sources had xenia effects in chestnut, and the results suggested proper species DBH and YL as pollination trees for YSZF.

**Key words:** *Castanea mollissima*, fruit quality and productivity, fruit set, yield, xenia.

**Wang J, Guo SJ. 2018.** Influences of different pollen sources on fruit quality and productivity of chestnut (*Castanea mollissima* Blume) in China. Taiwan J For Sci 33(4):263-75.

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Received January 2018, Accepted July 2018. 2018年1月送審 2018年7月通過。

## 研究報告

## 不同花粉授粉對中國板栗結實特性和果實品質的影響

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## 摘要

中國板栗種植面積和產量居世界第一，是世界上重要的果樹之一。同時，板栗也是中國最重要的木本糧食樹種之一，授粉對果實的品質以及結實能力起著至關重要的作用。試驗選用「燕山早豐」為母本，8個品種花粉為試材進行雜交授粉試驗，自然授粉為對照組，研究不同品種花粉對果實品質和果實結實特性的影響。板栗具有隔年結實特性，因此在研究板栗結實特性時取2年平均值作為最終試驗結果。在雜交實驗中，以「紫珀」為父本授粉後，果實著果率最高為86.67%，以「大板紅」為父本授粉後，產量(1.62 kg tree<sup>-1</sup>)最高，「遷西壯栗」為父本結實率(54.71%)最高，以「燕山早豐」為父本即自交組合結實率最低。與雜交組合相比，自交的單粒重較大。「紫珀」為父本時，果實中脂肪含量最低，粗蛋白含量最高；「遷西早紅」為父本時，可溶性糖含量最高；「燕山短枝」為父本時，果實中支鏈澱粉含量最高；「大板紅」為父本時，果實中直鏈澱粉含量最高。使用灰色關聯法對果實品質和果實結實特性進行綜合排序，以「大板紅」和「燕龍」為父本果實綜合評價最高，自交組合綜合評價最低，自然授粉次之。本研究不僅驗證了花粉直感效應對板栗果實品質和結實特性的影響，同時也為燕山早豐的授粉樹配置提供了理論依據。

關鍵詞：中國板栗、果實品質和結實特性、著果率、產量、花粉直感效應。

王靜、郭素娟。2018。不同花粉授粉對中國板栗結實特性和果實品質的影響。台灣林業科學33(4):263-75。

## INTRODUCTION

The chestnut (*Castanea mollissima* Blume) is a deciduous tree that belongs to the Fagaceae family and is native to China (Zhang et al. 2011, Zou et al. 2015a). It has been widely used as a food and timber crop in northern China since ancient times (Payne et al. 1983, Zhang et al. 2005). The chestnut can be used as a source of gluten-free flour since it contains essential dietary nutrients and minerals (Bounous and Marinoni 2005, Semih and Llknur 2012), and it has become an increasingly important crop in human nutrition. Chestnut is also an important nut species among fruit crops worldwide. The world area of cultivated chestnut is 602,718 ha, and China with 326,479 ha surpasses all other

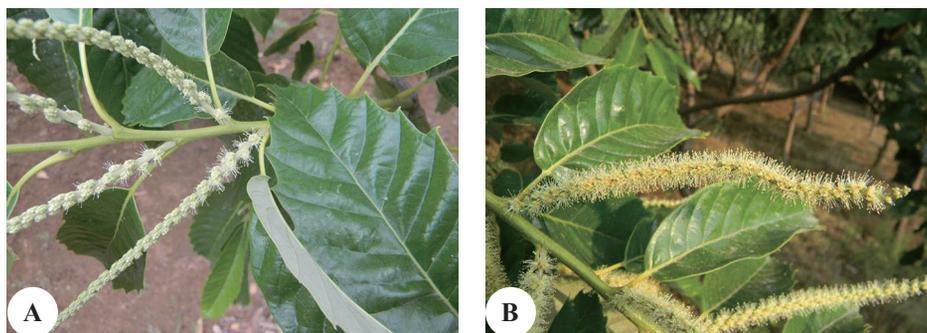
countries. The world production of chestnut was estimated to be more than 2 million t, with China being the main producer contributing 83.08% to the total yield (1,879,031 of 2,261,589 t, FAOSTAT 2016). Chestnut trees are distributed in over 24 provinces in southern and northern China. In many hilly areas of China, the chestnut can be the principle source of income for many rural families (Shi and Stösser 2005). In general, chestnut can both provide healthy, nutritious food to humans and serve as a major source of income for farmers (Martín et al. 2012, Zou et al. 2013, 2015a). In addition, the chestnut is considered a gene center for the genus *Castanea* (Vavilov 1951).

Chestnut is hermaphroditic, with both male and female flowers on the same tree. Chestnut is characterized by catkins and can be divided into unisexual flowers (Fig. 1) and bisexual flowers (Fig. 2). Unisexual flowers are male flowers, sessile, monochlamydeous, with 5~7 male flowers in a cluster, with slight filaments. Bisexual flowers are mixed inflorescences; the top of the inflorescence consists of male flowers, while the bottom has 1~3 female flowers. The female flower has a narrow-neck bottle configuration and is sessile (Zhang et al. 2011).

Chestnut is a self-incompatible (and wind pollinated) and cross-pollinated plant. The chestnut self-fertilization rate is < 1% (Mckay 1942). Self-pollination can seriously reduce chestnut production (Guo et al. 2013,

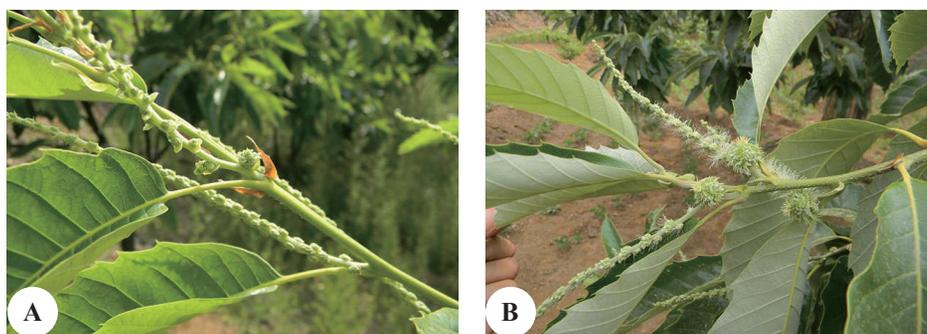
Lv et al. 2013). Chestnuts exhibits a set of floral features that tends to promote outcrossing (Klinac et al. 1995). Cross-pollination with other cultivars has to be provided to ensure satisfactory yields (Shi and Stösser 2005).

Fruit development of chestnut can be influenced by pollen, through the ‘xenia effect’, which was first described by Focke (1881). The xenia effect can cause changes in fruit form and size (xenoplasm) and in fruit color (xeno-chroms). Xenia provides a theoretical basis for the pollination tree configuration, fruit quality improvement, and advances in economic returns. Based on the importance of xenia, many scholars have studied its mechanism, including changes in endogenous hormone contents, isoenzyme variations, molecular biology, and so on. So far, there is no



Note: A. Initial flowering stage. B. Full-bloom stage.

**Fig. 1. Male catkin of chestnut.**



Note: Initial flowering stage. B. Full-bloom stage.

**Fig. 2. Male and female mixed inflorescence of chestnut.**

consistent conclusion about the mechanism of xenia (Yang 2012). The xenia effect was shown in studies on cross-pollination in almond (Kumar and Das 1996). Pollen sources have an effect on seeds and fruits in many crop species (Denney 1992), such as chestnut (McKay and Crane 1939), pear (Nyeki 1972), pistachio (Crane and Iwakiri 1980), apple (Ruth and Williams 1983), and pecan (Marquard 1988). In McKay and Crane's (1939) study, hybridization experiments with 3 species of chestnut showed that 2 different pollen sources produced nuts of different sizes with mean weights of 18.7 and 27.1 g, respectively, on the same tree.

The aim of this study was to evaluate the degree to which pollination by different cultivars influences both fruit productivity and fruit quality of chestnut (*C. mollissima* Blume).

## MATERIALS AND METHODS

### Plant materials, pollination, and experimental design

This study was carried out at the experimental base of Beijing Forestry Univ. (Qianxi County, Hebei Province, China), during the growth seasons (May to September) of 2 con-

secutive years, 2014 and 2015. The elevation of the experimental base is 163 m (118°12'E, 40°21'N), and the area is semi-humid, with a mean annual precipitation of 744.7 mm and a mean annual temperature of 10.9°C (Zou et al. 2013, Guo and Zou 2014). The soil is rich, with available N, P, and K contents of 75, 24, and 234 mg kg<sup>-1</sup>, respectively (Li et al. 2013).

Twelve-year-old chestnut trees (*C. mollissima*, 'Yanshanzaofeng' (YSZF)), planted at a spacing of 3×4 m, were used as female parents. Pollen was obtained from a germplasm nursery in Qianxi County, Hebei Province, China. Table 1 introduces 8 cultivars (Wang et al. 2008, Hebei Province Forestry Bureau 2016), and 7 cultivars were selected as pollinizers for the cross-pollination treatment: 'Qianxizaohong' (QXZH), 'Qianxizhuangli' (QXZL), 'Yanlong' (YL), 'Yankui' (YK), 'Zipo' (ZP), 'Dabanhong' (DBH), and 'Yanshanduanzhi' (YSDZ). We selected female trees of YSZF for the self-pollination treatment. Natural pollination by CK served as the control experiment.

The experiment was conducted in a completely randomized block design with 8 treatments (pollen sources), 3 replicates (3 female trees per plot), and a total of 72 trees each year in Qianxi County, Hebei Province, China.

**Table 1. Information of 8 chestnut (*Castanea*) cultivars**

Cultivars	Type	Number	Soluble sugars (%)	Starch (%)	Crude protein (%)
<i>C. Yanshanzaofeng</i> (YSZF)	cultivar	JiS-SV-CM-002-2005	19.69	51.34	4.43
<i>C. Qianxizaohong</i> (QXZH)	cultivar	JiS-SV-CM-014-2013	19.5	49.3	6.96
<i>C. Qianxizhongli</i> (QXZL)	cultivar	JiR-SV-CM-001-2013	21.5	49.6	5.89
<i>C. Yanlong</i> (YL)	cultivar	JiS-SV-CM-003-2009	22.6	48.2	6.01
<i>C. Yankui</i> (YK)	cultivar	JiS-SV-CM-001-2005	21.2	51.98	3.72
<i>C. Zipo</i> (ZP)	cultivar	JiS-SV-CM-001-2004	37.6 (total sugars)	38.26	23.22 (protein)
<i>C. Dabanhong</i> (DBH)	cultivar	JiS-SV-CM-004-2005	20.44	61.22	4.82
<i>C. Yanshanduanzhi</i> (YSDZ)	cultivar	JiS-SV-CM-003-2005	20.57	50.85	5.89
Qianxi 15	Superior individual				

To prevent uncontrolled pollination, clusters of unopened female flowers were covered with paper bags. Male catkins containing pollen from the 8 cultivars targeted for testing were harvested at a germplasm repository in late May and early June. Hand pollination was carried out in June (1~3 June 2014, and 7~9 June 2015) at 04:00~06:00. The female flower clusters were then re-covered with the paper bags until all male flowers in the experimental plots had withered and were no longer fertile (Zou et al. 2015b).

### Measurements and data collection

In early July of each year—1 mo after the experimental pollination in early June—the number of fruits that had set was recorded for each female flower cluster per tree. Fruits were harvested for further analysis in early autumn after burs had begun to open. The following data were collected: (1) number of fruits in each bur; (2) single fruit weight (g); and (3) total yield (kg). These productivity data were analyzed as follows: (1) fruit set percentage (%) as  $(\text{number of fruit} \times 100) / (\text{number of flowers pollinated})^{-1}$ ; (2) kernel percentage (%) as  $(\text{fruit weight} \times 100) / (\text{total weight})^{-1}$ ; and (3) total yield per tree (kg) as the kernel percentage (%)  $\times$  total bur weight (g)  $\times 1000^{-1}$ .

Further, fruit quality was estimated by calculating contents of fat, soluble sugars, crude protein, amylose, and amylopectin. Stored samples (1.000 g) were used to determine the fat content using a Soxhlet apparatus for 6 h (AOAC 1990). Total soluble sugar was determined by the anthrone colorimetric method (ultraviolet spectrophotometer at a 620-nm absorbance wavelength) with defatted samples (0.200 g) (Zou et al. 2015a). To determine the amylose and amylopectin contents in the samples, we used dual-wavelength spectrophotometry (0.200 g), with an ultra-

violet spectrophotometer to scan all bands, run absorbance curves for amylose and amylopectin, and confirm the amylose and amylopectin maximum absorbance wavelengths,  $\lambda_1$  and  $\lambda_3$ . We used isoabsorptive point mapping to confirm the amylose and amylopectin absorbances of  $\lambda_2$  and  $\lambda_4$ , and used  $\Delta\lambda = |\lambda_1 - \lambda_2|$  as the ordinate and the content of amylose (mg) as the abscissa to draw a standard amylose curve. Through use of the standard curve, we calculated the content of amylose. The content of amylopectin was calculated the same as for amylose. The level of crude protein was determined by the content of N\*6.25, while the content of N was measured using Auto Analyzer 3 (AA3 High Resolution). Three replicates were performed for each treatment in these assays.

### Statistical analysis

Duncan's multiple-range test and Pearson's correlation coefficient test were run using SPSS 21.0 software (SPSS, Chicago, IL, USA). Means were first compared by Duncan's multiple-range test at 0.05, and then Pearson's correlation coefficients were calculated at 0.01 and 0.05. The GRA method was used to select high-quality pollination trees by ranking them using a comprehensive set of fruit productivity and quality characteristics. The GRA methodology, developed on the basis of gray system theory (Deng 1989), has been broadly applied to evaluate intrinsic interrelationships among multiple variables (Hu et al. 2016). The GRA was used in this study with each treatment considered one of the factors in the GRA, and the gray relational grade (GRG) was obtained to assess the relationship between pollen sources and fruit quality parameters. (1) To normalize comprehensive fruit characteristics,  $Y_{ij}$ , is expressed as  $Y_{ij} = X_{ij} / X_{0j}$ , where  $X_{ij}$  is the original parameter,  $i = 1, 2, \dots, m$ , and  $j = 1, 2, \dots, n$ ; and  $X_{0j}$  is the

ideal treatment, defined as the highest value of any single parameter. Other treatments were defined as comparison treatments,  $X_1 \sim X_8$ . In this study,  $m$  is the number of pollen sources, and  $n$  is the number of comprehensive fruit characteristics. (2) The gray relational coefficient,  $\lambda_{ij}$ , was calculated as follows:  $\lambda_{ij} =$

$$\frac{\min_i \min_j |y_i^0 - y_{ij}| + \rho \max_i \max_j |y_i^0 - y_{ij}|}{|y_i^0 - y_{ij}| + \rho \max_i \max_j |y_i^0 - y_{ij}|}, \text{ where } y_i^0$$

is the ideal normalized result for the  $i$ th parameter; and  $\rho$  is a distinguishing coefficient, which can be set from 0 to 1, and was set as 0.5 in this study (Wang et al. 2015). (3) The GRG was used in this study to assess relationships between a pollen source and all recorded fruit characteristics, and was carried out using the equation  $R_i = \sum_{j=1}^m w_j \lambda_{ij}$ , where  $R_i$  is the GRG for the  $j$ th treatment;  $w_j$  is the weighting factor for the  $i$ th parameter, and  $m$  is the number of fruit quality parameters. The GRA was calculated using Excel 2013 (Microsoft, Redmond, WA, USA).

## RESULTS

### Fruit productivity

An analysis of combined data from this

2-yr study revealed that the pollen source significantly influenced the fruit set, total yield, number of fruits per bur, kernel percentage, and single-grain weight (Table 2). Because of alternate bearing in chestnut, the average of results from 2 yr is also shown in Table 2.

### Fruit set (%) and total yield (kg tree<sup>-1</sup>):

In this study, the pollen source significantly ( $r = -0.389$ ,  $p < 0.01$ ) influenced the average fruit set over 2 yr (Table 2). Fruit set of the recipient YSZF trees ranged from a low of 38.89% (with YK pollen in 2014) to the highest value of 97.78% (with DBH pollen in 2015), whereas self-pollination resulted in the lowest fruit set of 15.56% in 2014 and 22.22% in 2015. Averaging data of 2 yr resulted in the highest fruit set of 86.67% with ZP pollen, while the lowest was 18.89% with self-pollination. Fruit set of natural pollination was in the middle range.

The total yield significantly ( $r = -0.317$ ,  $p < 0.05$ ) differed among pollen sources (Table 2), ranging from 1.07 (with YSDZ in 2014) to 2.10 kg tree<sup>-1</sup> (by DBH in 2015). Self-pollination resulted in the lowest yield 0.53 and 0.59 kg tree<sup>-1</sup> in 2014 and 2015, respectively. Self-pollination resulted in the lowest yield 0.56 kg tree<sup>-1</sup> in a 2-yr average, while DBH pollen

**Table 2. Effects of 8 pollen sources on chestnut fruit productivity in 2014 and 2015**

Pollen source	Fruit set (%)			Total yield (kg tree <sup>-1</sup> )			Fruits bur <sup>-1</sup> (N)			Kernel percentage (%)			Single-grain weight (g)		
	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.
QXZH	77.78 <sup>cd</sup>	94.44 <sup>ab</sup>	86.11 <sup>a</sup>	1.21 <sup>fgh</sup>	1.24 <sup>fgh</sup>	1.23 <sup>a</sup>	2.57 <sup>a</sup>	2.47 <sup>ab</sup>	2.52 <sup>a</sup>	48.50 <sup>def</sup>	50.79 <sup>d</sup>	49.65 <sup>abc</sup>	5.34 <sup>fg</sup>	7.08 <sup>cd</sup>	6.21 <sup>a</sup>
QXZL	61.11 <sup>f</sup>	90.00 <sup>b</sup>	75.56 <sup>a</sup>	1.21 <sup>fgh</sup>	1.73 <sup>bc</sup>	1.47 <sup>a</sup>	2.27 <sup>ab</sup>	2.57 <sup>a</sup>	2.42 <sup>a</sup>	47.40 <sup>def</sup>	62.01 <sup>a</sup>	54.71 <sup>a</sup>	4.98 <sup>gh</sup>	6.74 <sup>de</sup>	5.86 <sup>a</sup>
YL	72.22 <sup>de</sup>	91.11 <sup>ab</sup>	81.67 <sup>a</sup>	1.19 <sup>fgh</sup>	1.86 <sup>b</sup>	1.53 <sup>a</sup>	2.63 <sup>a</sup>	2.63 <sup>a</sup>	2.63 <sup>a</sup>	49.97 <sup>de</sup>	54.36 <sup>c</sup>	52.17 <sup>ab</sup>	4.22 <sup>hi</sup>	8.68 <sup>ab</sup>	6.45 <sup>a</sup>
YK	38.89 <sup>g</sup>	58.89 <sup>f</sup>	48.89 <sup>b</sup>	1.13 <sup>gh</sup>	1.56 <sup>cd</sup>	1.35 <sup>a</sup>	2.53 <sup>a</sup>	2.27 <sup>ab</sup>	2.40 <sup>a</sup>	46.61 <sup>ef</sup>	57.43 <sup>bc</sup>	52.02 <sup>ab</sup>	3.85 <sup>i</sup>	6.82 <sup>cd</sup>	5.35 <sup>a</sup>
ZP	82.22 <sup>c</sup>	91.11 <sup>ab</sup>	86.67 <sup>a</sup>	1.15 <sup>gh</sup>	1.36 <sup>ef</sup>	1.26 <sup>a</sup>	2.07 <sup>cd</sup>	1.67 <sup>cd</sup>	1.87 <sup>b</sup>	43.03 <sup>g</sup>	46.35 <sup>efg</sup>	44.69 <sup>c</sup>	4.57 <sup>ghi</sup>	8.62 <sup>ab</sup>	6.60 <sup>a</sup>
DBH	75.56 <sup>cd</sup>	97.78 <sup>a</sup>	82.78 <sup>a</sup>	1.13 <sup>gh</sup>	2.10 <sup>a</sup>	1.62 <sup>a</sup>	2.63 <sup>a</sup>	2.4 <sup>ab</sup>	2.52 <sup>a</sup>	49.93 <sup>de</sup>	58.01 <sup>b</sup>	53.97 <sup>ab</sup>	4.65 <sup>ghi</sup>	7.24 <sup>cd</sup>	5.95 <sup>a</sup>
YSDZ	58.89 <sup>f</sup>	90.00 <sup>b</sup>	74.45 <sup>a</sup>	1.07 <sup>h</sup>	1.30 <sup>fg</sup>	1.19 <sup>a</sup>	2.63 <sup>a</sup>	2.33 <sup>ab</sup>	2.48 <sup>a</sup>	48.35 <sup>def</sup>	46.78 <sup>ef</sup>	47.57 <sup>bc</sup>	5.13 <sup>fgh</sup>	7.82 <sup>bc</sup>	6.48 <sup>a</sup>
YSZF	15.56 <sup>h</sup>	22.22 <sup>h</sup>	18.89 <sup>c</sup>	0.53 <sup>i</sup>	0.59 <sup>i</sup>	0.56 <sup>b</sup>	0.30 <sup>d</sup>	0.37 <sup>d</sup>	0.34 <sup>c</sup>	31.82 <sup>h</sup>	28.12 <sup>i</sup>	29.97 <sup>d</sup>	6.04 <sup>ef</sup>	8.82 <sup>a</sup>	7.43 <sup>a</sup>
CK	65.67 <sup>ef</sup>	73.44 <sup>d</sup>	69.56 <sup>a</sup>	1.10 <sup>gh</sup>	1.52 <sup>de</sup>	1.31 <sup>a</sup>	2.07 <sup>bc</sup>	1.70 <sup>b</sup>	1.89 <sup>b</sup>	45.44 <sup>f</sup>	48.02 <sup>de</sup>	46.73 <sup>bc</sup>	4.50 <sup>ghi</sup>	6.39 <sup>de</sup>	5.45 <sup>a</sup>

In each column, means followed by different superscript letters significantly differ at the 0.01 level according to Duncan's multiple-range test.

Pollen sources are defined in Table 1.

resulted in the highest yield of 1.62 kg tree<sup>-1</sup>. There was a negative correlation ( $r = -0.582$ ,  $p < 0.01$ ) between the pollen source and the total yield in 2014, and a negative correlation ( $r = -0.317$ ,  $p < 0.05$ ) for the average of 2 yr (Table 3). There were positive correlations ( $r = 0.792/2014$ ,  $r = 0.690/2015$ ,  $r = 0.750/avg.$ ,  $p < 0.01$ ) between fruit set and total yield (Table 3).

**Number of fruits bur<sup>-1</sup>:** The number of fruits bur<sup>-1</sup> was higher with cross-pollination (by 1.67 to 2.63) than with self-pollination (by 0.30 to 0.37) (Table 2). Fruits bur<sup>-1</sup> by natural pollination was in a middle range. Negative correlations ( $r = -0.486/2014$ ,  $p < 0.01$ ,  $r = -0.640/2015$ ,  $r = -0.551/avg.$ ,  $p < 0.01$ ) were found between the pollen source and fruits bur<sup>-1</sup>. Fruits bur<sup>-1</sup> was positively correlated with fruit set ( $r = 0.703/2014$ ,  $r = 0.857/2015$ ,  $r = 0.692/avg.$ ,  $p < 0.01$ ) and total yield ( $r = 0.888/2014$ ,  $r = 0.752/2015$ ,  $r = 0.613/avg.$ ,  $p < 0.01$ ) (Table 3).

**Kernel percentage (%):** In this study, kernel percentage was negatively affected by the pollen source ( $r = -0.465/2014$ ,  $r = -0.556/2015$ ,  $r = -0.483/avg.$ ,  $p < 0.01$ ); values ranged from a low of 28.12% (with self-pollination in 2015) to the highest of 62.01% (by QXZL in 2015) (Table 2). Kernel percentages for YL, YK, DBH, and QXZL, which ranged 54.36–62.01%, were significantly

greater than those with pollen sources from QXZH, ZP, and YSDZ and natural pollination in 2015. After averaging 2014 and 2015 data, the QXZL pollen source had the highest kernel percentage with 54.71%. Self-pollination resulted in the lowest kernel percentages of 31.82% in 2014, 28.12% in 2015, and 29.97% on average. The kernel percentage was positively correlated with fruit set ( $r = 0.712/2014$ ,  $r = 0.694/2015$ ,  $r = 0.719/avg.$ ,  $p < 0.01$ ), total yield ( $r = 0.891/2014$ ,  $r = 0.877/2015$ ,  $r = 0.874/avg.$ ,  $p < 0.01$ ), and fruits bur<sup>-1</sup> ( $r = 0.951/2014$ ,  $r = 0.865/2015$ ,  $r = 0.801/avg.$ ,  $p < 0.01$ ) (Table 3).

**Single-grain weight (g):** The single-grain weight of chestnut was not significantly affected by the pollen source (Table 2). The highest single-grain weights, 6.04 and 8.82 g, were observed during 2014 and 2015 with self-pollination. The single-grain weight with natural pollination was next to last in 2014 and last in 2015. Natural pollination also was next to last in the 2-yr averages. Pollen sources of YL, YSDZ, and ZP resulted in heavier grain weights in 2015. The crossing treatment with pollen of YL and ZP showed higher single-grain weights and significant differences compared to those from the pollen of QXZH, QXZL, YK, DBH, and YSDZ ( $p < 0.05$ ) in 2015, but the pollen of YL and ZP did not show a higher single-grain weight compared

**Table 3. Pearson's correlation coefficient for chestnut fruit productivity**

	Pollen source			Fruit set			Total yield			Fruit number			Kernel percentage		
	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.	2014	2015	Avg.
Fruit set	-0.371	-0.465*	-0.389**	1	1	1									
Total yield	-0.582**	-0.287	-0.317*	0.792**	0.690**	0.750**	1	1	1						
Fruit number	-0.468*	-0.640**	-0.551**	0.703**	0.857**	0.692**	0.888**	0.752**	0.613**	1	1	1			
Kernel percentage	-0.465*	-0.556**	-0.483**	0.712**	0.694**	0.719**	0.891**	0.877**	0.874**	0.951**	0.865**	0.801**	1	1	1
Single-grain weight	0.134	0.109	0.062	-0.332	-0.247	0.191	-0.474*	-0.369	0.257	-0.539**	-0.348	-0.290*	-0.473*	-0.607**	-0.021

Data from both study years are combined.

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

to the other pollen sources in 2014. In 2014, self-pollination resulted in a higher single-grain weight than did cross-pollination ( $p < 0.05$ ). In the 2-yr average, self-pollination had the highest single-grain weight, while the YK pollen source had the lowest weight. The single-grain weight was negatively correlated with fruits  $\text{bur}^{-1}$  ( $r = -0.539/2014$ ,  $p < 0.01$ ,  $r = -0.290/\text{avg.}$ ,  $p < 0.05$ ), total yield ( $r = -0.474/2014$ ,  $p < 0.05$ ), and kernel percentage ( $r = -0.473/2014$ ,  $p < 0.05$ ,  $r = -0.607/2015$ ,  $p < 0.01$ ) (Table 3). The single-grain weight was not correlated with the pollen source, fruit set, total yield, or kernel percentage for the 2-yr average.

### Fruit quality

Analyses of fruit quality indicators showed that the pollen source played an important role in fat, soluble sugar, crude protein, amylose, and amylopectin contents (Table 4).

**Fat (%):** Self-pollination resulted in the greatest fat percentage in 2014/2015 (5.11/5.03%) compared to cross-pollination treatments (Table 4). ZP pollen had significantly

lower fat (4.17/1.64%) compared to the other treatments in both seasons. The fat percentage of natural pollination was in the middle. Interestingly, the fat percentage in 2014 was higher than that in 2015 from the same pollen sources. There was no significant relationship between fat contents and the pollen source (Table 5).

**Soluble sugars (%):** Natural pollination resulted a middle range of soluble sugar contents. QXZH pollen resulted in the highest percentages of soluble sugars in 2014/2015 (22.02/34.76%), while self-pollination resulted in the lowest soluble sugar percentages (13.97/21.12%). The soluble sugar content had negative correlations with the pollen source ( $r = -0.421/2014$ ,  $p < 0.05$ ;  $r = -0.471/2015$ ,  $p < 0.05$ ), and with fat ( $r = -0.395/2014$ ,  $p < 0.05$ ) (Table 5).

**Crude protein (%):** In 2014, ZP pollen resulted in the highest crude protein content (9.38%) among all treatments, whereas in 2015, self-pollination resulted in the highest content (9.20%). Natural pollination resulted in the lowest crude protein contents in 2014/2015 (7.24/5.71%). Crude protein

**Table 4. Effects of 8 pollen sources on chestnut fruit quality in 2014 and 2015**

Pollen source	Fat (%)		Soluble sugars (%)		Crude protein (%)		Amylose (%)		Amylopectin (%)	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
QXZH	4.72 <sup>ab</sup>	4.56 <sup>abc</sup>	22.02 <sup>bcde</sup>	34.76 <sup>a</sup>	7.98 <sup>d</sup>	6.19 <sup>f</sup>	18.06 <sup>b</sup>	13.65 <sup>ghi</sup>	31.92 <sup>bcde</sup>	31.05 <sup>cde</sup>
QXZL	4.65 <sup>abc</sup>	3.96 <sup>cd</sup>	18.66 <sup>cde</sup>	31.37 <sup>ab</sup>	7.43 <sup>e</sup>	6.41 <sup>f</sup>	14.26 <sup>fgh</sup>	14.85 <sup>defg</sup>	37.03 <sup>a</sup>	33.31 <sup>abcd</sup>
YL	4.88 <sup>a</sup>	4.11 <sup>bcd</sup>	16.54 <sup>de</sup>	24.10 <sup>abcde</sup>	8.46 <sup>cd</sup>	9.03 <sup>ab</sup>	16.12 <sup>bcdefg</sup>	11.93 <sup>hi</sup>	33.18 <sup>abcd</sup>	23.40 <sup>f</sup>
YK	4.67 <sup>abc</sup>	4.11 <sup>bcd</sup>	16.46 <sup>de</sup>	28.40 <sup>abc</sup>	8.94 <sup>abc</sup>	6.40 <sup>f</sup>	18.05 <sup>b</sup>	14.41 <sup>efgh</sup>	30.47 <sup>de</sup>	27.71 <sup>ef</sup>
ZP	4.17 <sup>bcd</sup>	1.64 <sup>e</sup>	18.03 <sup>cde</sup>	28.66 <sup>abc</sup>	9.38 <sup>a</sup>	7.11 <sup>c</sup>	15.14 <sup>cdefg</sup>	17.21 <sup>bcde</sup>	33.13 <sup>abcd</sup>	31.80 <sup>bcde</sup>
DBH	4.75 <sup>ab</sup>	3.83 <sup>d</sup>	15.72 <sup>de</sup>	25.05 <sup>abcde</sup>	8.72 <sup>bc</sup>	6.36 <sup>f</sup>	21.41 <sup>a</sup>	16.88 <sup>bcdef</sup>	35.67 <sup>abc</sup>	25.71 <sup>f</sup>
YSDZ	4.66 <sup>abc</sup>	3.8d	17.29 <sup>cde</sup>	25.84 <sup>abcd</sup>	8.81 <sup>bc</sup>	7.34 <sup>e</sup>	14.54 <sup>defgh</sup>	15.41 <sup>bcdefg</sup>	36.36 <sup>ab</sup>	32.84 <sup>abcd</sup>
YSZF	5.11 <sup>a</sup>	5.03 <sup>a</sup>	13.97 <sup>e</sup>	21.12 <sup>bcde</sup>	8.00 <sup>d</sup>	9.20 <sup>ab</sup>	18.81 <sup>bc</sup>	17.35 <sup>bcd</sup>	34.23 <sup>abcd</sup>	32.25 <sup>bcde</sup>
CK	4.75 <sup>ab</sup>	4.05 <sup>bcd</sup>	16.46 <sup>de</sup>	26.64 <sup>abcd</sup>	7.24 <sup>e</sup>	5.71 <sup>g</sup>	15.16 <sup>cdefg</sup>	11.53 <sup>i</sup>	30.35 <sup>de</sup>	24.56 <sup>f</sup>

In each column, means followed by different superscript letters significantly differ at the 0.05 level according to Duncan's multiple-range test.

Pollen sources are defined in Table 1.

**Table 5. Pearson’s correlation coefficient for chestnut fruit quality**

	Pollen source		Fat		Soluble sugars		Crude protein		Amylose		Amylopectin	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Fat	0.170	0.014	1	1								
Soluble sugars	-0.421*	-0.471*	-0.395*	-0.010	1	1						
Crude protein	-0.48	0.109	-0.358	0.179	-0.074	-0.432*	1	1				
Amylose	-0.13	0.166	0.166	-0.275	-0.026	-0.159	0.190	0.169	1	1		
Amylopectin	-0.45	-0.143	0.198	-0.099	-0.351	0.156	0.055	0.031	-0.182	0.530**	1	1

Data from both study years are combined.

\* Significant at  $p < 0.05$ ; \*\* significant at  $p < 0.01$ .

showed a negative correlation with soluble sugars ( $r = -0.432/2015, p < 0.05$ ) (Table 5).

**Amylose and amylopectin (%):** QXZL pollen resulted in the highest amylopectin contents in 2014/2015 (37.03/33.31%), but low amylose contents (14.26/14.58%) (Table 4). Natural pollination resulted in the lowest amylose content in 2015 (11.53%) and amylopectin contents in 2014 and 2015 (30.35/24.56%). The same pollen sources resulted in higher amylopectin contents in 2014 than in 2015. A positive correlation was ob-

served between amylose and amylopectin ( $r = 0.530/2015, p < 0.01$ ) (Table 5). Amylose and amylopectin were not significantly correlated with each other.

**Rankings of comprehensive fruit productivity and fruit quality by the GRA**

Referring to the results of the GRA (Table 6) method, because natural pollination (CK) was next to last, it is necessary to configure pollination for chestnut trees. Cross-pollination with pollen sources of DBH and

**Table 6. Ranking of comprehensive chestnut fruit productivity and fruit quality by the gray relational analysis (GRA) method, and correlation coefficient and relational degree of all treatments**

	Y <sub>1</sub>	Y <sub>2</sub>	Y <sub>3</sub>	Y <sub>4</sub>	Y <sub>5</sub>	Y <sub>6</sub>	Y <sub>7</sub>	Y <sub>8</sub>	Y <sub>9</sub>	Y <sub>10</sub>	R <sub>j</sub>	Rank of R <sub>j</sub>
λ <sub>1</sub>	0.98	0.62	0.90	0.81	0.72	0.82	1.00	0.67	0.69	0.79	0.83	4
λ <sub>2</sub>	0.75	0.81	0.83	1.00	0.66	0.72	0.77	0.65	0.62	1.00	0.84	3
λ <sub>3</sub>	0.87	0.88	1.00	0.89	0.76	0.77	0.58	1.00	0.59	0.67	0.86	2
λ <sub>4</sub>	0.47	0.70	0.82	0.89	0.59	0.74	0.65	0.76	0.72	0.69	0.68	7
λ <sub>5</sub>	1.00	0.64	0.65	0.68	0.82	0.48	0.69	0.87	0.72	0.84	0.80	5
λ <sub>6</sub>	1.00	1.00	0.90	0.97	0.67	0.72	0.58	0.74	1.00	0.75	0.94	1
λ <sub>7</sub>	0.73	0.59	0.87	0.75	0.76	0.70	0.62	0.84	0.64	0.96	0.76	6
λ <sub>8</sub>	0.33	0.37	0.31	0.46	1.00	1.00	0.51	0.96	0.83	0.87	0.52	9
λ <sub>9</sub>	0.66	0.68	0.58	0.73	0.60	0.75	0.62	0.63	0.56	0.64	0.67	8
w <sub>y</sub>	0.29	0.21	0.09	0.15	0.06	0.02	0.03	0.02	0.04	0.11		

Y<sub>1</sub>, fruit set; Y<sub>2</sub>, total yield; Y<sub>3</sub>, fruits bur<sup>-1</sup>; Y<sub>4</sub>, kernel percentage; Y<sub>5</sub>, single-grain weight; Y<sub>6</sub>, fat percentage; Y<sub>7</sub>, soluble sugar percentage; Y<sub>8</sub>, crude protein percentage; Y<sub>9</sub>, amylose percentage; Y<sub>10</sub>, amylopectin percentage; λ<sub>1</sub>~λ<sub>9</sub>, correlation coefficients of pollen sources (QXZH, QXZL, YL, YK, ZP, DBH, YSDZ, self-pollination, CK, respectively); R<sub>j</sub>, weighted correlation; w<sub>y</sub>, final weighting by the AHP method.

Pollen sources are defined in Table 1.

YL ranked first and second, which means that choosing DBH and YL as pollination trees can result in higher fruit productivity and better fruit quality. In this study, self-pollination was last, a manifestation of chestnut self-incompatibility.

## DISCUSSION

### Fruit productivity

It is well known that successful fruit set in chestnut depends on both pollination and subsequent fertilization. Results of fruit set in the 2 seasons studied (Table 2) agreed with the findings of other authors (Fattahi et al. 2014). In a 2-yr average, self-pollination also had the lowest fruit set, total yield, fruits bur<sup>-1</sup>, kernel percentage, and highest single-grain weight. The reduced fruit set with self-pollination can be explained by self-incompatibility, a well-known phenomenon in chestnut. Fruit sets caused by all pollen sources were higher in 2015 than in 2014, which may have been caused by biennial fruiting. Self-incompatibility can seriously reduce chestnut production (Lv et al. 2013). In their study, self-pollination had the lowest yield. Their study also configured pollination trees for YSZF, and they selected Qianxi 15 and DBH as pollination trees for YSZF. Results of the total yield also showed that the same pollen source (DBH) led to higher yields.

Results of the number of fruits bur<sup>-1</sup> showed that cross-pollination produced more fruits bur<sup>-1</sup> than did self-pollination in 2014 and 2015, and for the 2-yr average. Self-pollination resulted in more unfertilized burs than did cross-pollination, which might have been a result of self-incompatibility. The kernel percentage is one of the most important parameters used in evaluating variables such as yield, variety, and pollination tree configuration. This result is similar to those shown

in previous studies on hazelnut and almond (Hosseini-Ava et al. 2006, Bahmani et al. 2002). Single-grain weights in all treatments were lower in 2014 than in 2015, which might have been an effect of the biennial bearing of fruit or environmental conditions. In 2014, a lack of rain in August influenced the fruit-expansion process. Through a correlation analysis in Table 3, a significant or highly significant relationship existed among the pollen source, fruit set, total yield, fruits bur<sup>-1</sup>, and kernel percentage in 2-yr average values.

### Fruit quality

Results of fat contents showed that chestnut has a low fat content (Table 4). This characteristic has led to its increased use in low-calorie diets (Anonymous 2012, Ertürk et al. 2006). Soluble sugar percentages change from 1 season to the next. Contents of soluble sugars in 2015 were generally higher than those with the same treatments in 2014 (Table 4). A higher protein content is an important parameter in chestnut production in terms of essential amino acids (Morrone et al. 2015). ZP, QXZH, and YSZF pollen resulted in the highest crude protein contents in 2014 and 2015. Starch is the main constituent of chestnuts (Borges et al. 2008, Liu et al. 2015, Pereira-Lorenzo et al. 2006), and is determined by assaying amylose and amylopectin contents. QXZL pollen resulted in the highest amylopectin contents in 2014 and 2015, while natural pollination resulted in the lowest amylose content in 2015 and amylopectin contents in 2014 and 2015.

### Rankings of comprehensive fruit productivity and fruit quality by the GRA

In chestnut production, farmers expect to get higher yields and improved quality to boost incomes. The GRA method had a score ranking to synthetically quantify and evaluate

the influence of different pollen sources on chestnut fruit productivity and fruit quality parameters. DBH and YL were selected as pollination trees for YSZF.

## CONCLUSIONS

It is known that the pollen source affects fruit productivity and fruit quality in several kinds of nut crops. Proper selection of pollen sources has the potential to modify and improve fruit production from year to year as shown in previous studies. In this study, DBH and YL pollen sources ranked best in comprehensive fruit characteristics according to the GRA, whereas self-pollination ranked last. Thus, it can be concluded that pollen sources have xenia effects in chestnut, and self-pollination and cross-pollination influence the fruit quality and productivity. Moreover, this study helped choose DBH and YL as pollination trees for YSZF, a leading chestnut cultivar in China.

## ACKNOWLEDGEMENTS

The authors would like to thank the helpful comments and suggestions of the teachers and colleagues in the Key Laboratory for Silviculture and Conservation, Ministry of Education, Beijing Forestry Univ. We also thank Beijing Forestry Univ. for providing technical support in determining fruit quality. We also acknowledge support for the project by the Special Funds of National Forestry Public Welfare Major Foundation of China (grant no. 201204401) and Twelfth Five-Year Plan in National Science Key Technology of Non-wood Cultivation (grant no. 2012BAS1B0402).

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