

Article

The Efficiency of Artificial Pollination on the Hazelnut ‘Tonda Francescana’[®] Cultivar and the Xenia Effects of Different Pollinizers

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Abstract

Pollination is a determining factor in achieving economic yield in hazelnut cultivation, and together with variable climate conditions, this requires the use of artificial pollination. This study evaluated the efficiency of artificial pollination performed with a manual sprayer using pollen from three pollinizer cultivars on the ‘Tonda Francescana’[®] commercial orchard and the effect of different pollen sources on nuts. Dry pollens were applied by a Pollen Blower machine twice during female blooming. The pollen of ‘Nocchione’ determined the highest fruit set and yield per tree, even if it did not determine the highest blank seed percentage. The open pollinizers exhibited a lower sphericity and shape index (NSI), ‘Camponica’ pollen was associated with the biggest nut and kernel; ‘San Giovanni’ pollen showed higher nut elongation. Artificial pollination turned out to be a good tool to increase yield, but its efficiency is strongly influenced by the pollen used.

Keywords: fruit set; kernel percentage; kernel weight; kernel width; nut elongation; nut shape index

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1. Introduction

Pollination is essential for the production of most fruit and nut crops, yet it is often a limiting factor for both yield and product quality [1]. However, especially in nut crops, there is still limited knowledge regarding the variation in fruit and seed production in anemophilous and monoecious species with diclinous flowers, such as *Corylus avellana* L.

and *Juglans regia* L. [2]. Although considerably smaller in terms of global production, the tree nut industry is rapidly expanding. Among nut crops, the European hazelnut (*Corylus avellana* L.) is a species of significant interest for its nutritional value, with global cultivation covering more than 660,000 hectares worldwide [3]. However, both the yield and quality of these agricultural commodities can be severely limited by inadequate pollination. Therefore, significant efforts in the science and management of pollination are urgently warranted [1]. Hazelnut is a monoecious wind-pollinated species, meaning that it has separate male and female flowers, has diclinous flowers (Charts 1 and 2), and is subjected to a strong inter-annual yield fluctuation [4]. This phenomenon, which is common to other fruit crops, is known as alternate or biennial bearing and consists in a higher fruit production in the ON year which inhibits flowering in the OFF year, hence leading to lower fruit load. Some authors [4] have shown that yield and pollen production are significantly related, and author [5] underlined how climate change, with phenological shifts—such as in pollination, anthesis, fertilization, and nut set—can disrupt the synchrony of pollen release and female flower receptivity, reducing pollination efficiency. In fact, for instance, asynchronous pollen release and female flower receptivity and adverse weather conditions (e.g., heavy rainfall, lack of pollinizer) during the pollination period can significantly diminish the likelihood of successful pollination. That said, to avoid inter-annual yield fluctuation it is necessary to ensure the presence of pollen in the hazelnut, even artificially [6–8]. Hazelnut exhibits sporophytic self-incompatibility, with its anemophilous pollination occurring during winter under conditions of low temperatures and high relative humidity, while the formation of ovules begins in March and fertilization occurs by the end of May or during the first three weeks of June, four to five months after pollination, when the diameter of the nuts is 7–10 mm [9–11]. Moreover, male and female flowers ripened and bloomed at different time. In hazelnuts, the most important horticultural traits, such as nut weight, kernel percentage and shell thickness, are highly heritable, and pollen sources could increase nut and kernel weight and decrease blank percentage [12]. A high level of flower cluster drop may be observed before fertilization, in late April or May. This drop is dependent on the apical dominance which exists along one-year-old shoots and along the peduncle of catkins. The formation of seedless nuts is a complex phenomenon. When fertilization does not occur or when the kernel does not grow enough after fertilization, blank fruits may appear [13]. Beyond genetic and cultural factors, some climatic parameters could play a major role in these phenomena [9]. So, considering that, to date, almost all cultivated varieties of hazelnut exhibit self-incompatibility, and that the edible part is the seed, namely the kernel, pollination and fertilization are obligatory in hazelnuts for the growth of the embryo and for the ovule to develop. Without fertilization, the embryo does not grow and neither does the seed; thus, incompatibility and the dichogamy mechanism can bring the selection of pollinizer cultivars forward [6,13].

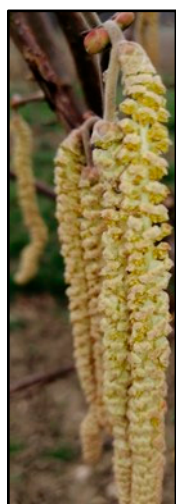


Chart 1. Male inflorescences at full blooming.

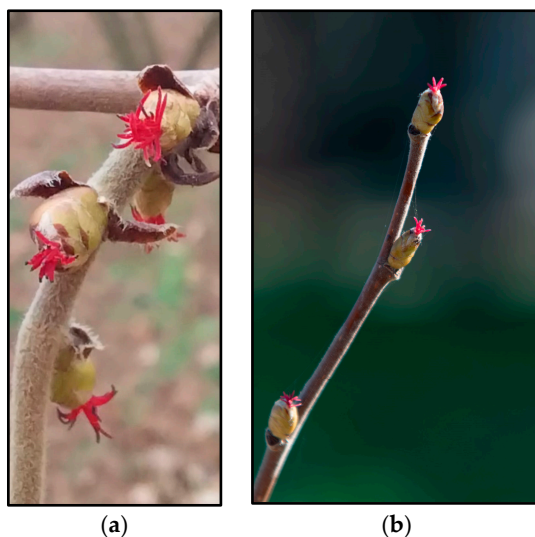


Chart 2. Female glomerulus (a) and single female flowers in bloom (b) (by A. Ottaviani).

Hazelnut production is susceptible to interannual yield variations, which negatively affect price predictability in the market [2,5,11]. An excessive fruit load can compromise the onset of flowering, resulting in reduced production in the following cycle [4]. However, this study highlighted that the correlations between yields and pollen peaks were positive, while the ones with pollen season duration were negative, indicating that a short and intense male flowering is beneficial to fruit production [4]. However, pollination is a determining factor in achieving economic yield in hazelnut cultivation. The source and quantity of pollen are essential practices in orchard management, directly impacting fruit production and quality [14,15]. Pollination is a critical factor in fruit production and is highly dependent on wind; however, these systems can be affected by changes such as variations in flowering and susceptibility to climatic events. In this context, artificial pollination becomes an important tool for improving pollination efficiency and fruit set [6–8]. This method has proven effective and has been tested in various fruit species, including kiwi, olive, passion fruit [16,17] and hazelnuts [18]. Artificial pollination can increase fruit yield and fill pollination gaps in some fruit crops, and its application to hazelnuts is still not well explored. Some tests have been carried out in new countries, such as Chile and South Africa, outside the native range of the species, i.e., Europe and western Asia, where hazelnut is experiencing new agro-environmental conditions in terms of climatic variability and pedologic characteristics, which threaten the

pollination process and hamper nut yield [4,18,19]. Climate change poses multifaceted challenges to hazelnut cultivation, impacting various stages of growth and development, from floral differentiation and blossom to pollination and nut setting. Changes in climatic conditions, especially temperatures, influence the initiation and progression of the phenological growth stages in all the areas of hazelnut cultivation [5,20]. In Italy, the second-highest-producing country in the world, after Turkey, the production of hazelnuts in recent years has been strongly affected by climate change, which has also affected the production of pollen or reduced the synchrony of pollen release and female flower receptivity, with the reduction in pollination efficiency [20]. In order to try to have a higher pollination efficiency, it is also necessary to evaluate the possibility of resorting to the artificial distribution of pollen for hazelnuts, as has already performed for other species with wind pollination, such as walnuts [21]. Pollen can be applied either dry or wet using different equipment, such as vibratory technologies that do not require external pollen, dry application technologies that require pure pollen, and wet application technologies that involve a carrier solution along with the pollen [6]. In hazelnuts, artificial pollination has shown promising results, particularly in regions with unfavorable climatic conditions. Research indicates that artificial pollination can increase fruit production, although pollen viability varies among different cultivars [22]. Pollinizers influence fruit shape; therefore, when selecting pollinizing plants, it is crucial to consider compatibility to avoid changes in fruit characteristics [14,22,23]. These changes, known as the xenia effect, affect fruit morphology, leading to variations in shape, size, and kernel weight [14,24,25]. Pollinating cultivars can alter the biochemical composition of the fruits [25] and the act of pollinating plants is essential not only for determining productivity but also for ensuring commercial fruit quality [12].

This study aimed to (a) evaluate the efficiency of artificial pollination performed with a manual sprayer using pollen from three pollinizer cultivars ('Camponica', 'Nocchione', and 'San Giovanni'), compared to open pollination, on 'Tonda Franciscana®' commercial orchard; (b) assess the paternal effects on the number of normal seeds per nut, as well as evaluate the influence of different pollen sources on both the quantity and quality (xenia) of the production of the 'Tonda Franciscana®' cultivar; and (c) investigate the relationship between nut shape parameters considering the different pollinizer cultivars.

2. Materials and Methods

2.1. Plant Material

The study was carried out on 6-year-old trees of the 'Tonda Franciscana®' cultivar, cultivated in a commercial orchard of the Fondazione per l'Istruzione Agraria in Perugia (Italy), located in the Umbria region (42°57'22" N, 12°23'45" E). The hazelnut orchard, 1 hectare large, with 9 rows, is managed with irrigation and the plants are grown as single stems. The planting distance is 3.5 m between plants and 5 m between rows. The commercial cultivars 'Camponica', 'Nocchione' and 'San Giovanni' were selected as pollinizers, not considering, at this stage, the pollen shed and pistillate flower emergence but only pollinizer compatibility, based on literature references [26–29], considering that the pollen was going to be artificially sprayed. 'Camponica' and 'Nocchione' have already been tested as pollenizers of 'Tonda Franciscana®' [12] in the same environment as this research, and results showed they were suitable pollenizers; 'San Giovanni' pollen was used since it was the only one commercially available.

2.2. Pollen Collection

The pollens of the ‘Camponica’ and ‘Nocchione’ cultivars were collected in an orchard near that of the test while the pollen of ‘San Giovanni’ was harvested in the Campania region (Italy). The artificial pollination was compared to the wind pollination (later open pollination or control) by counting flowers, fruit set and fruits collected from wind-pollinated plants located in the same orchard [26–29] but far enough away to not receive artificially distributed pollen. In the orchard, there are only two other cultivars beyond the main ‘Tonda Franciscana’®, one row of ‘Tonda Gentile Romana’ and one of ‘Tonda di Giffoni’ cultivars, which are the parents of ‘Tonda Franciscana’® cultivars [12]. This hazelnut orchard is isolated, being about 1 km away from other orchards, which are also separated by tree plant barriers. When catkins of the selected pollinizers were about to shed, pollen was collected and stored in a vial in the freezer. The pollen was collected by the Pollen AspiraPollenMini2 machine (Biotac, Verona, Italy) (Chart 3). This machine consists of two 4-meter hoses, which are light and antistatic, to avoid electrostatic effects on the pollen. It is equipped with its own motor and is designed for easy transportation, featuring two wheels and telescopic legs that facilitate mobility in the field. The machine has no metal components that meet the pollen so its viability is not altered in any way. The pollen vitality was evaluated as reported by [30] and it was around 50%, in agreement with that reported for ‘Camponica’ and ‘Nocchione’ cultivar by [10].



Chart 3. Pollen AspiraPollenMini2 machine (Biotac, Verona—Italy).

2.3. Experimental Design and Pollen Application

Artificial pollination was applied on three long rows, with 60 plants each, and one row was kept as the control and was wind pollinated. On four trees per each pollinated row, four small branches, with an average of 15 ± 0.89 flower glomeruli per branch, were selected for the counting of flowers and the setting, and were identified with wire tags. The male flowers (catkins) were not removed prior to female anthesis due to sporophytic self-incompatibility that occurs in hazelnut [2,19,27,31].

Female flower glomeruli at full anthesis were pollinated twice, the first application on 19 February 2024 and the second ones on 27 February 2024, by applying pollen to the stigma with a hand sprayer, using pollen only, with no additives or inert excipients. More specifically, the application distance from target flowers during spraying ranged from 0.5 to 1.0 m. Two pollen applications were performed to cover the whole female blooming period, which spanned from February 10 to the end of February, when there are still female flowers in full bloom, that is, when more than 50% of the stigmas are fully extended [32].

The machine used to blow pollen is the Pollen Blower machine (Biotac, Verona—Italy) and is an instrument capable of distributing dry pollen (Chart 4). It consists of a blower with an internal combustion engine, a pollen micro-dosing dispenser and a rechargeable battery (Table 1). Through a flow of air at a maximum speed of 600 m³/h, pollen is sprayed onto the plant continuously. The pollen distribution of the micro-doser is adjustable.

Table 1. Technical specifications of Pollen Blower machine (<https://www.biotac.it>, accessed on 15 May 2025).

Technical Characteristics	Technical Specifications
Pollen delivers	100–800 g/h
Support backpack weight	5.5 kg
Application speed	7–8 km/h
Air flow maximum speed	600 m ³ /h

During flowering, the operator walks under the plants, directing the air jet towards the flowers (Chart 3). For pollination, 0.8 g per plant of pollen was used, equal to 48 g ha^{−1} per application. This quantity was chosen because it was between that used by other researchers, which values ranged from 30 to 150 g ha^{−1} [18,33]. The artificial pollination was applied twice to cover, as much as possible, the whole female blooming period of ‘Tonda Franciscana®’, which is usually two–three weeks long [34].

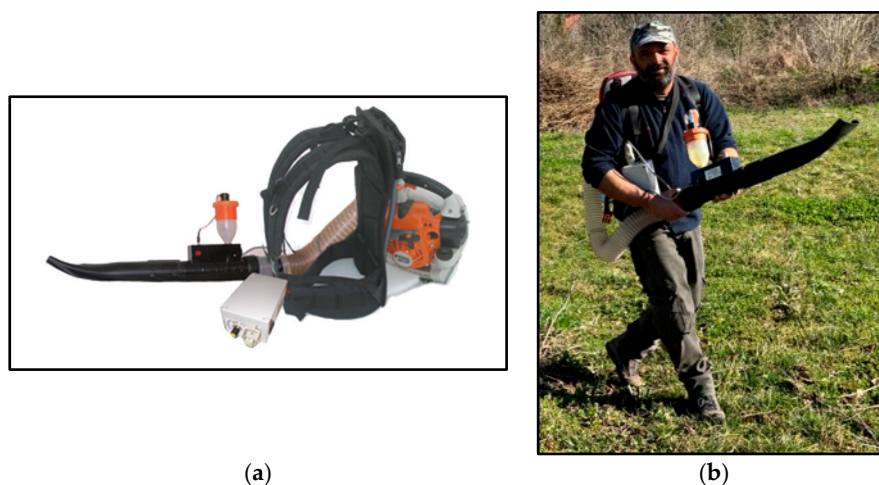


Chart 4. Hand sprayer (a) (by biotac.it) and operator with hand sprayer (b) (by A. Ottaviani).

The artificial pollinations were carried out around 12:00 a.m., with an air temperature of 9 °C, air humidity of 65–75%, and when the wind speed was very low or at 0 km h^{−1} to avoid pollen contamination. The meteorological data were collected using a Spectrum (Thayer Court, Aurora, IL, USA) WatchDog 2000 Series Weather Station located close to the orchard.

2.4. Field Recording Data

At the beginning of June, the labeled branches, 16 per pollinizer, were bagged with a net-bag to collect all nuts, even those which eventually drooped prior to full ripening (Figure 1, Chart 5).

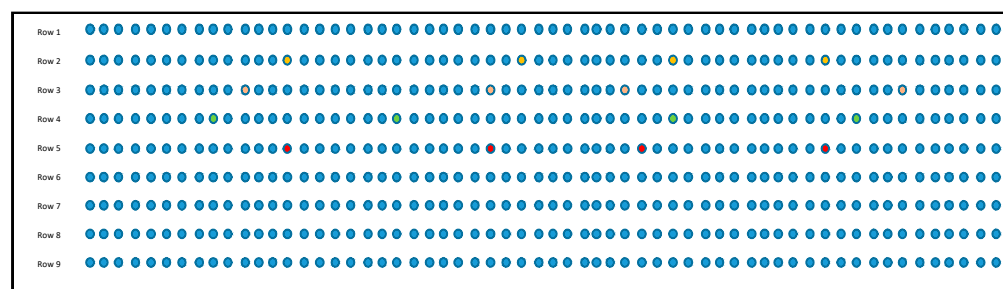


Figure 1. Layout schematic illustrating the positions of experimental sample collection points, indicated with circles of different colors; the blue circles represent the plants in the hazelnut orchard; the orange circles represent the sampled trees wind-pollinated; the pink circles indicate those pollinized with pollen of ‘Camponica’; the green circles those pollinized with pollen of ‘Nocchione’ and the red ones those pollinized with pollen of ‘San Giovanni’.



Chart 5. Fruiting branch of ‘Tonda Franciscana®’ at the beginning of June (a) and nut cluster (b).

At the end of August, a total of 1542 nuts were collected from the bagged branches. For each branch, fruit setting was expressed as the number of fruit per female flower glomeruli or cluster. The following nut characteristics were also determined: the percentage of blank nuts; of nuts with one well developed seed and of nuts with two, or double, seeds; the number of nuts with no viable seeds; the percentage with one or two viable seeds; nut and kernel weight [12]. Moreover, each row was harvested by a harvester machine (FACMA) to determine yield.

For each nut sample and per each pollinizer, all the collected nuts were used to measure nut and kernel traits. Nut samples were dried to a constant mass prior to all measurements; the dryer used was a small-sized curing cell (De Cloet S.r.l., Città di Castello, Italy), at the “Nut-living lab” managed by the University of Perugia.

2.5. Nut and Kernel Measurements

Each nut of each sample was weighed (nut weight—NW) by digital balance (PA64C OHAUS Corporation, Tempcon Instrumentation, Arundel, UK); then, after breaking each with a bench clamp, shells (shell weight—SW) and kernels (kernel weight—KW) were weighed too. Moreover, it was noted if there were no seeds, one seed or double seeds for each nut.

The percent kernel (PK) was calculated as the ratio of kernel weight and nut weight $\times 100$.

The changes that occurred in the embryo, endosperm and all maternal tissue through the pollen effect, namely ‘xenia’, were evaluated by measuring the following nut and kernel characteristics [6]:

The nut shape index (NSI) [35] was calculated as the following ratio:

$$NSI = \frac{WI+T}{2L}$$

where WI means nut width, T nut thickness, and L nut length.

The Kernel content (KC) in relation to nut volume was calculated as the following:

$$\frac{NW \times PK}{V \times 100}$$

where KC denotes kernel fill.

Three linear dimensions (Figure 2) of nut length (L), width (WI) and nut thickness (T), were all measured in mm with calipers (Brand Kmt®tools, Brookhouse Industrial Estate, Stoke-on-Trent, UK) [36].

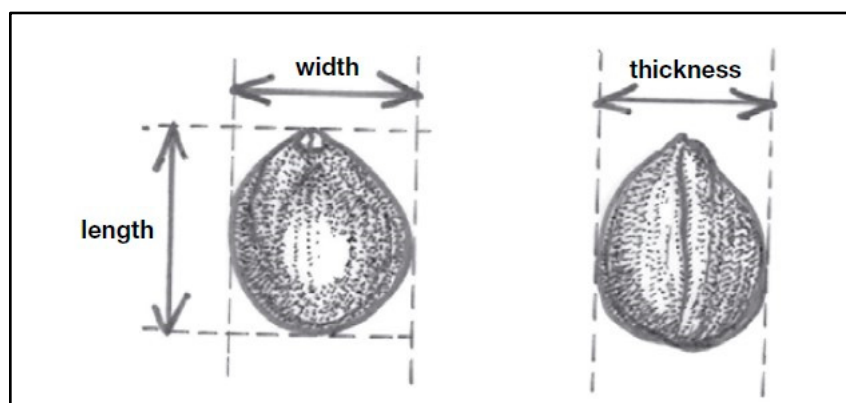


Figure 2. Nut length (L), nut width (WI) and nut thickness (T) [36].

In accordance with what was suggested by [35], the following traits have been calculated:

Elongation (E) was calculated by using the following relationship:

$$E = \frac{\text{major axis length}}{\text{min or axis length}}$$

Using the three linear dimensions of nuts (L, WI, T), arithmetic mean diameter (Da), geometric mean diameter (Dg) and sphericity (ϕ /SPH) were defined as follows:

$$Da = \frac{L+WI+T}{3}$$

$$Dg = \sqrt[3]{LWI T}$$

$$\Phi/SPH = \frac{Dg}{L}$$

The aspect ratio (Ra) (%) was calculated as follows:

$$Ra = WI/L \times 100$$

The surface area (S), in cm², was calculated as follows:

$$S = \pi Dg^2$$

The nut volume (NV), in cm³, was calculated as follows:

$$NV = \pi LWIT/6$$

Finally, to determine how homogeneous the size of kernel was, the percentage of kernels falling in different diameter classes was calculated, this being a good measure of homogeneity, e.g., the % of kernels in the class from 12 to 14 mm in diameter.

The diagram of samplings is given in Figure 3.

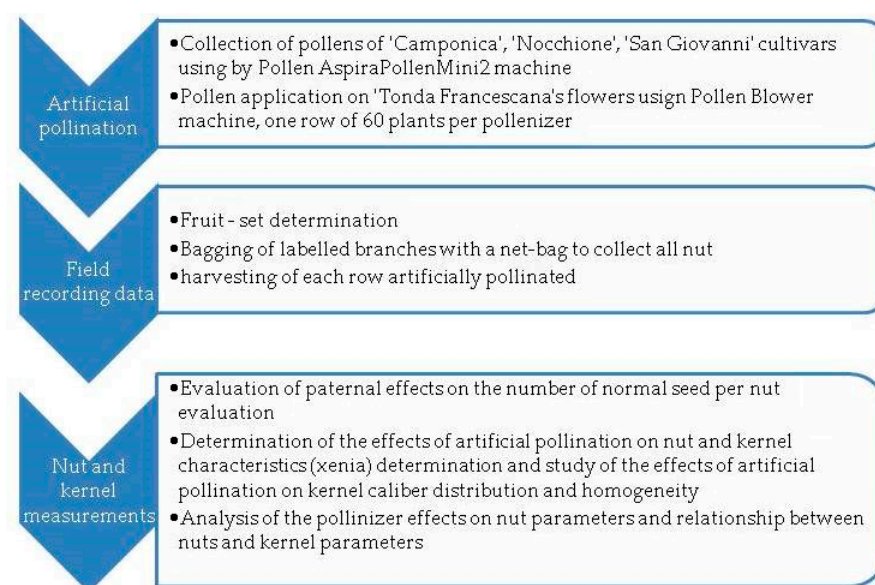


Figure 3. Diagram of samplings.

2.6. Statistical Analysis

The effect of all possible pollination combinations among the cultivar 'Tonda Francescana®' and other pollinizers on the number and quality of seeds and their development, in addition to nut and seed characteristics, were evaluated using the chi square test (χ^2) of independence. Specifically, the χ^2 test of independence was used to test the significance of the relationship between rows (seed pattern) and columns (pollen) in a contingency table. In each column (pollen) the "expected" frequencies were calculated by multiplying the total number of seeds by the percentage expected to be in the category (undeveloped seed, one normal seed and two normal seeds) [37]. Since the samples came from a normal distribution, the frequencies observed should be close to the expected frequencies based on a normal distribution. For each distribution, an χ^2 test was performed to test whether the observed frequencies differed significantly from the expected frequencies.

The data were statistically evaluated by one-way ANOVA to assess the significance of the main factor (pollinizers) and a Duncan test was used to determine the significance of differences among the means, with the significance level at $p \leq 0.05$. The relationships among variables were examined using Pearson's correlation analysis at a significance level of $p < 0.05$ using the Infostat software version 2020 [38].

Principal component analysis (PCA) was performed using nut and kernel characteristics as input variables to explore the variability among pollinizers and to detect the most discriminating variables. PCA summarizes the information contained in the data matrix in fewer independent PCs, obtained as linear combinations of the original variables, lying in the direction of maximum variance [39]. The data were statistically evaluated using R (R Core Team, 2018).

3. Results

3.1. Effect of Artificial Pollination on Fruit Set

The cultivar 'Nocchione' achieved the highest fruit set among all pollinizers and under open pollination, increasing the fruit set by 22.81% compared to wind pollination (Table 2). On the contrary, the pollens of the cultivars 'Camponica' and 'San Giovanni' resulted in a fruit set not different from that obtained with uncontrolled pollination (Table 2).

Table 2. Fruit set for ‘Tonda Franciscana’® at harvest, expressed as number of fruits per female flower glomeruli after pollination with four different cultivars (means $\pm$ s.e. accompanied by different letters are significantly different at $p \leq 0.05$).

Pollinizers	Fruit Set (n. Fruit/Female Flower Glomeruli)
Camponica	2.28 ± 0.26 b
Nocchione	2.94 ± 0.16 a
San Giovanni	2.25 ± 0.10 b
Open pollination (Control)	2.39 ± 0.13 b

Mean values followed by different letters are significantly different at $p < 0.05$.

3.2. Effects of Artificial Pollination on Yield

The effects of artificial pollination on yield were also evaluated, and these depended on the pollinizer used (Figure 3). In fact, the pollen, artificially sprayed, of ‘Nocchione’ had the highest yield per tree, expressed as kilograms of nut in shell, significantly higher than that obtained by open pollination; on the contrary, the pollens of ‘Camponica’ and ‘San Giovanni’ showed less effective results (Figures 4 and 5). More specifically, the pollen of ‘Nocchione’ exhibited a yield increase of 26.3%, while those of ‘Camponica’ and ‘San Giovanni’ showed a yield decrease of 9.2% and 38.4%, respectively.

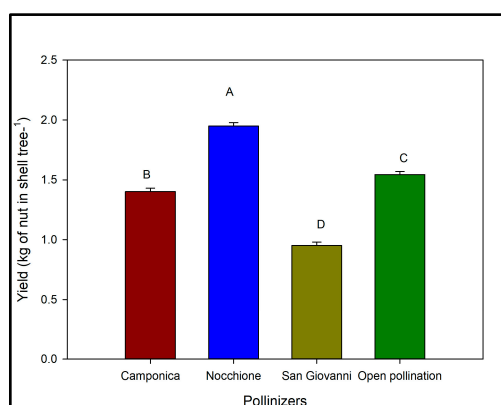


Figure 4. Yield as kilograms of nut of Tonda Franciscana® in shell tree⁻¹, obtained from different pollinizers (means $\pm$ s.e. followed by different letters are significant different per $p < 0.05$).

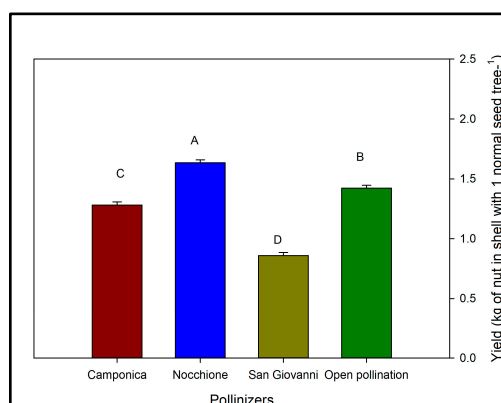


Figure 5. Yield as kilograms of nut of Tonda Franciscana® in shell with 1 normal seed tree⁻¹, obtained from different pollinizers (means $\pm$ s.e. followed by different letters are significant different per $p < 0.05$).

3.3. Paternal Effects on the Number of Normal Seeds per Nut

Even considering the percentage of hazelnuts with undeveloped seeds, reported in Table 2, expressed also as blank seeds, a negative qualitative parameter, the artificial pollination by ‘Nocchione’ resulted in the most effective increase in the yield of ‘Tonda Francescana®’ (Figure 4).

Data on seed pattern showed that there was a significant paternal effect (pollen) on the number of undeveloped seeds per nut (Table 2).

Artificial pollination between ‘Nocchione’, as the pollinizer, and ‘Tonda Francescana®’ gave rise to a higher percentage of undeveloped seeds: 15.46% compared to the 9.27% expected for that category (overall $\chi^2 = 22.01$, $p \leq 0.1$).

On the other hand, no pollinizer has, however, caused a change in the percentage of double seeds (Table 3).

Table 3. Contingency table (4×3) showing paternal effects on number of normal seeds per nut in ‘Tonda Francescana®’ (as mother cultivar).

Seed Pattern	Pollination Treatments	Camponica		Nocchione		San Giovanni		Open Pollination		Percent/Total
		N. of Seed	Percent/Total	N. of Seed	Percent/Total	N. of Seed	Percent/Total	N. of Seed	Percent/Total	
Undeveloped (blank) seed	Observed	9	7.76	45	15.46	66	9.23	39	6.58	9.27
	Expected	10.75		26.98		66.29		54.98		
	Cell χ^2	0.29		<u>12.04</u>		0.00		4.64		
One normal seed	Observed	106	91.38	244	83.85	646	90.35	546	92.07	89.91
	Expected	104.3		261.65		642.87		533.18		
	Cell χ^2	0.03		1.19		0.02		0.31		
Double seeds	Observed	1	0.89	2	0.69	3	0.42	8	1.35	0.82
	Expected	0.95		2.38		5.84		4.84		
	Cell χ^2	0.00		0.06		1.38		2.06		

Overall $\chi^2 = 22.01$ ($p < 0.1$). Underlined number: higher χ^2 cell value.

3.3.1. Effects of Artificial Pollination on Nut Characteristics (Xenia)

The fruit characteristics, related to the weight of fruit components, were affected by artificial pollination (Table 4). More specifically, ‘Camponica’ and ‘Nocchione’ as pollinizers showed nuts (2.81 g and 2.79 g, respectively), kernels (1.30 g and 1.32 g, respectively) and shells (1.49 g for both) heavier than those produced by ‘San Giovanni’ and ‘control’. No significant differences were present among the pollinizers with respect to the open pollination for the percentage kernel, which varied from 46.57 to 47.07 (Table 4).

Table 4. Effect of artificial pollination on weight of fruit components (means $\pm$ s.e.).

Pollinizers	Nut Weight (NW) (g)	Kernel Weight (KW) (g)	Shell Weight (SW) (g)	Percentage Kernel (PK)
Camponica	2.81 $\pm$ 0.09 a	1.30 $\pm$ 0.04 ab	1.49 $\pm$ 0.05 a	46.64 $\pm$ 0.50 a
Nocchione	2.79 $\pm$ 0.07 a	1.32 $\pm$ 0.03 a	1.49 $\pm$ 0.04 a	47.07 $\pm$ 0.35 a
San Giovanni	2.65 $\pm$ 0.04 ab	1.23 $\pm$ 0.02 bc	1.41 $\pm$ 0.02 ab	46.57 $\pm$ 0.21 a
Open pollination (Control)	2.53 $\pm$ 0.05 b	1.18 $\pm$ 0.02 c	1.34 $\pm$ 0.03 b	46.71 $\pm$ 0.28 a

Per each column, mean values followed by different letters are significantly different per $p < 0.05$.

3.3.2. Effects of Artificial Pollination on Fruit and Kernel Shape (Xenia)

Nut width, nut thickness, and nut shape index were affected by pollen sources (Table 5). The ‘Camponica’ pollinizer exhibited the widest ‘Tonda Francescana®’ nut, as well as the highest thickness; the nut shape index ranged from 0.91 to 0.93, meaning a round nut [40], and ‘Camponica’ determined the highest index (Table 5).

Table 5. Effect of artificial pollination on nut dimension and nut shape index (means $\pm$ s.e.).

Pollinizers	Nut Width (NWI) (mm)	Nut Thickness (NT) (mm)	Nut Length (NL) (mm)	Nut Shape Index (MSI)
Camponica	19.02 $\pm$ 0.17 a	16.98 $\pm$ 0.18 a	19.37 $\pm$ 0.20 a	0.93 $\pm$ 0.010 a
Nocchione	18.87 $\pm$ 0.12 ab	16.42 $\pm$ 0.13 b	19.49 $\pm$ 0.14 a	0.91 $\pm$ 0.004 b
San Giovanni	18.58 $\pm$ 0.10 b	16.28 $\pm$ 0.08 b	19.36 $\pm$ 0.09 a	0.90 $\pm$ 0.002 b
Open pollination (Control)	18.58 $\pm$ 0.07 b	16.22 $\pm$ 0.10 b	19.15 $\pm$ 0.11 a	0.91 $\pm$ 0.003 b

For each column, mean values followed by different letters are significantly different for $p < 0.05$.

‘Camponica’ affected the ‘Tonda Francescana®’s nut volume and nut surface, while no differences were exhibited for nut aspect ratio by the pollinizers (Table 6). ‘Nocchione’ influenced the kernel content, exhibiting the highest value of 0.42 (Table 6).

Table 6. Effect of artificial pollination on nut characteristics (means $\pm$ s.e.).

Pollinizers	Nut Volume (NV) (cm ³)	Nut Surface Area (NS) (cm ²)	Nut Aspect Ratio (NRa)	Kernel Content (KC)
Camponica	3.29 $\pm$ 0.08 a	10.67 $\pm$ 0.18 a	95.82 $\pm$ 1.1 a	0.40 $\pm$ 0.010 b
Nocchione	3.19 $\pm$ 0.06 ab	10.45 $\pm$ 0.13 ab	95.19 $\pm$ 0.8 a	0.42 $\pm$ 0.010 a
San Giovanni	3.01 $\pm$ 0.03 b	10.23 $\pm$ 0.08 b	95.41 $\pm$ 0.5 a	0.40 $\pm$ 0.004 ab
Open pollination (Control)	3.05 $\pm$ 0.04 b	10.12 $\pm$ 0.10 b	96.16 $\pm$ 0.6 a	0.39 $\pm$ 0.005 b

For each column, mean values followed by different letters are significantly different for $p < 0.05$.

‘Camponica’ influences the nut shape characteristics of ‘Tonda Francescana®’, exhibiting the longest arithmetic mean diameter as well as the highest geometric mean diameter and sphericity (Table 7). On the contrary, ‘Camponica’ exhibited the lowest elongation of nuts (Table 7).

Table 7. Effect of artificial pollination on nut shape (means $\pm$ s.e.)

Pollinizers	Nut Elongation (NE)	Nut Arithmetic Mean Diameter (NDa) (mm)	Nut Geometric Mean Diameter (NDg) (mm)	Nut Sphericity (NSPH)
Camponica	1.11 $\pm$ 0.02 b	18.46 $\pm$ 0.16 a	18.41 $\pm$ 0.16 a	0.95 $\pm$ 0.04 a
Nocchione	1.17 $\pm$ 0.01 a	18.28 $\pm$ 0.11 ab	18.21 $\pm$ 0.11 ab	0.93 $\pm$ 0.03 b
San Giovanni	1.18 $\pm$ 0.01 a	18.07 $\pm$ 0.07 b	18.01 $\pm$ 0.07 b	0.93 $\pm$ 0.03 b
Open pollination (Control)	1.17 $\pm$ 0.01 a	17.98 $\pm$ 0.09 b	17.92 $\pm$ 0.09 b	0.94 $\pm$ 0.02 b

For each column, mean values followed by different letters are significantly different for $p < 0.05$.

3.3.3. Effects of Artificial Pollination on Kernel Characteristics (Xenia)

More differences were noted between kernel width and kernel thickness (Table 8). ‘Camponica’, followed by ‘Nocchione’, exhibited the widest kernel as well as the thickest kernel, while ‘Nocchione’ showed the shortest kernel; finally, ‘Camponica’ had the least-elongated kernel (Table 8).

Table 8. Effect of artificial pollination on kernel dimensions (means $\pm$ s.e.)

Pollinizers	Kernel Width (KWI) (mm)	Kernel Thickness (KT) (mm)	Kernel Length (KL) (mm)	Kernel Elongation (KE)
Camponica	14.26 $\pm$ 0.19 a	12.45 $\pm$ 0.27 a	15.17 $\pm$ 0.25 ab	1.23 $\pm$ 0.03 b
Nocchione	13.99 $\pm$ 0.14 ab	11.81 $\pm$ 0.19 b	15.48 $\pm$ 0.18 a	1.33 $\pm$ 0.01 a
San Giovanni	13.68 $\pm$ 0.08 b	11.41 $\pm$ 0.12 b	15.01 $\pm$ 0.11 ab	1.33 $\pm$ 0.02 a
Open pollination (Control)	13.77 $\pm$ 0.11 b	11.76 $\pm$ 0.15 b	14.76 $\pm$ 0.14 b	1.28 $\pm$ 0.02 ab

For each column, mean values followed by different letters are significantly different for $p < 0.05$.

The highest kernel arithmetic mean diameter (KDa) and the highest geometric mean diameter (KDg) as well as the highest kernel surface area were produced by ‘Camponica’, followed by ‘Nocchione’ (Table 9). The kernel aspect ratio ranged from 91.02 to 95.39, without a significant difference between the pollinizers and the open pollination treatments (Table 9). The kernel sphericity ranged from 0.89 to 0.94, meaning round kernels, but the highest value was obtained by ‘Camponica’ followed by the ‘control’ (Table 9).

Table 9. Effect of artificial pollination on kernel dimension and shape (means $\pm$ s.e.).

Pollinizers	Kernel Arithmetic Mean Diameter (KDa) (mm)	Kernel Geometric Mean Diameter (KDg) (mm)	Kernel Aspect Ratio (KR _a)	Kernel Sphericity (KSPH)	Kernel Surface Area (KS) (cm ²)
Camponica	13.96 $\pm$ 0.17 a	13.87 $\pm$ 0.17 a	95.39 $\pm$ 2.1 a	0.92 $\pm$ 0.01 a	6.07 $\pm$ 0.14 a
Nocchione	13.76 $\pm$ 0.12 ab	13.64 $\pm$ 0.12 ab	91.02 $\pm$ 1.5 a	0.89 $\pm$ 0.01 b	5.87 $\pm$ 0.10 ab
San Giovanni	13.37 $\pm$ 0.07 c	13.25 $\pm$ 0.08 c	91.74 $\pm$ 0.9 a	0.89 $\pm$ 0.01 b	5.55 $\pm$ 0.06 c
Open pollination (Control)	13.43 $\pm$ 0.09 bc	13.34 $\pm$ 0.10 bc	93.94 $\pm$ 1.2 a	0.91 $\pm$ 0.01 ab	5.62 $\pm$ 0.08 bc

For each column, mean values followed by different letters are significantly different for $p < 0.05$.

3.3.4. Effects of Artificial Pollination on Kernel Caliber Distribution and Homogeneity

The kernel diameter distribution was slightly affected by pollen source (Figure 6). In fact, differences were noted only for two diameter classes: the class from 11.0 mm to 11.99 mm (caliber 11 mm in the figure) and the class from 14 mm to 14.99 mm (caliber 14 mm) (Figure 6). ‘San Giovanni’ and ‘Open pollination’ pollens showed the highest percentage of kernel, with diameters ranging from 11 to 1.99 mm—8.86% and 7.53%, respectively, while ‘Nocchione’ showed the lowest value of 2.9% (Figure 5). On the contrary, ‘Camponica’ showed the highest percentage of kernel with a diameter of 14 mm, equal to 34.7%, and ‘San Giovanni’ showed the lowest, equal to 25.6% (Figure 6).

Finally, the kernel homogeneity, evaluated as percentages of kernels with a caliber between 12 and 14 mm, and those with a caliber between 13 and 15 mm, was not influenced by pollen sources (Figure 7).

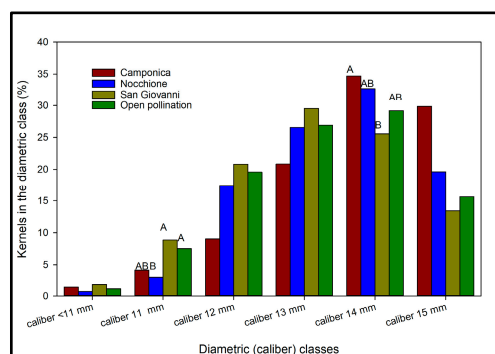


Figure 6. Kernel diameter distribution. For each diameter class means followed by different letters are significantly different at $p < 0.05$.

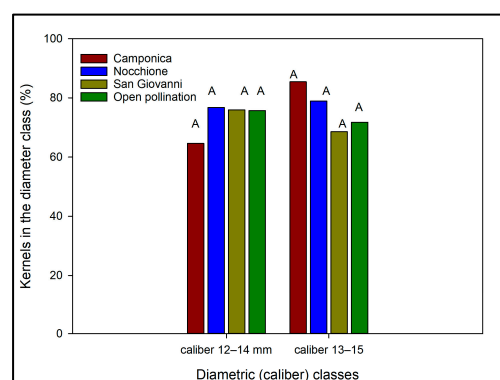


Figure 7. Kernel homogeneity. For each diameter class means followed by different letters are significant different at $p < 0.05$.

3.4. Pollinizer Effects on Nut Parameters

The PCA biplot (Figure 8) illustrates the distribution of the four pollination treatments based on nut and kernel characteristics observed for ‘Tonda Franciscana®’, where the first two principal components (PCs) explain 69.5% of the total variance.

Pollinizers positioned towards the right, specifically the open pollinizers, exhibited a lower sphericity and shape index (NSI). Conversely, the ‘Camponica’ pollinizer, positioned towards the left, is associated with the biggest nut and kernel (Figure 8). The ‘San Giovanni’ pollinizer, positioned on the bottom of the PCA biplot, showed higher nut elongation, while ‘Nocchione’ did not exhibit any important xenia effects on ‘Tonda Franciscana®’ fruits (Figure 8).

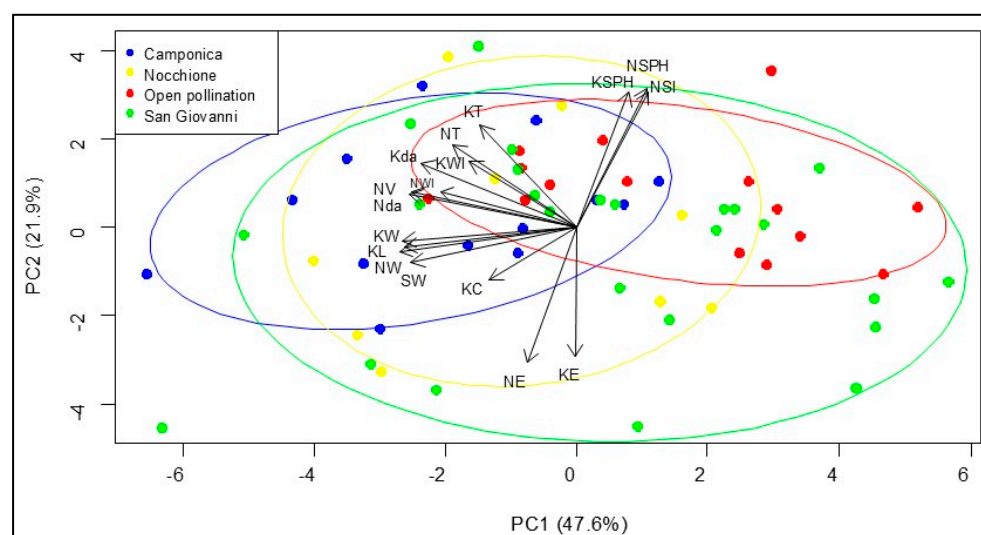


Figure 8. Scatter plots of PCA scores for three pollinizers and control based on nut and kernel characteristics. Biplots show the relationships between PC1 and PC2. Ellipses represent 95% confidence intervals around group centroids. Abbreviations used in Figure 8 are as follows: NSPH = Nut Sphericity; KSPH = Kernel Sphericity; NSI = Nut Shape Index; NT = Nut Thickness; KWI = Kernel Width; KDa = Kernel Arithmetic Mean Diameter; NWI = Nut Width; NV = Nut Volume; NDa = Nut Arithmetic Mean Diameter; KW = Kernel Weight; KL = Kernel Length; NW = Nut Weight; SW = Shell Weight; KC = Kernel Content; NE = Nut Elongation; KE = Kernel Elongation.

3.5. Relationship Between Nuts and Kernel Parameters

Pearson's correlations revealed strong positive relationships among traits related to nut and kernel size—including nut weight (NW), kernel weight (KW), shell weight (SW), nut volume (NV), and various dimensional parameters (e.g., nut and kernel length, width, and diameter). By contrast, shape-related descriptors, such as nut sphericity (NSPH) and nut shape index (NSI), showed negative correlations with elongation indices (NE, KE) in pairwise analysis (Table 10).

These patterns are reflected in the multivariate PCA results (Figure 8), where PC1 captures the overall variation in size-related traits and separates genotypes primarily based on differences in nut and kernel mass and dimensions. PC2, instead, accounts for variation in shape-related traits.

Table 10. Estimates of Pearson’s correlation coefficients among main physical nuts (then reported as a prefix as N) and kernels (then reported as a prefix as K) of ‘Tonda Franciscana®’. For abbreviations of variables, see the Material and Methods section. Underlined are non-significant values at the level of $p < 0.05$.

Variable	NW	SW	KW	PK	NWI	NT	NL	NSI	KC	NV	NE	NDa	NDg	NSPH	NRa	NS	KWI	KT	KL	KE	KDa	KDg	KSPH	KRa	KS
NW	1																								
SW	0.97	1																							
KW	0.96	0.88	1																						
PK	−0.23	−0.41	0.04	1																					
NWI	0.71	0.71	0.66	−0.25	1																				
NT	0.67	0.65	0.65	−0.19	0.52	1																			
NL	0.86	0.87	0.78	−0.37	0.64	0.73	1																		
NSI	−0.28	−0.32	−0.21	0.25	0.19	<u>0.05</u>	−0.50	1																	
KC	0.51	0.39	0.65	0.45	<u>0.07</u>	<u>−0.03</u>	<u>0.14</u>	−0.20	1																
NV	0.86	0.85	0.80	−0.30	0.81	0.88	0.91	<u>−0.11</u>	<u>0.07</u>	1															
NE	<u>0.07</u>	<u>0.10</u>	<u>0.03</u>	<u>−0.16</u>	<u>−0.17</u>	<u>−0.09</u>	0.36	−0.76	<u>−0.03</u>	<u>0.04</u>	1														
NDa	0.86	0.86	0.80	−0.31	0.82	0.87	0.91	<u>−0.11</u>	<u>0.08</u>	1.00	<u>0.04</u>	1													
NDg	0.86	0.85	0.80	−0.31	0.81	0.88	0.91	<u>−0.10</u>	<u>0.07</u>	1.00	<u>0.04</u>	1.00	1												
NSPH	−0.26	−0.28	−0.20	<u>0.18</u>	0.18	<u>0.12</u>	−0.45	0.97	−0.24	<u>−0.06</u>	−0.74	<u>−0.07</u>	<u>−0.06</u>	1											
NRa	−0.20	−0.21	−0.19	<u>0.04</u>	<u>−0.08</u>	<u>0.00</u>	<u>−0.01</u>	<u>−0.01</u>	−0.28	<u>−0.05</u>	0.64	<u>−0.05</u>	<u>−0.04</u>	<u>0.02</u>	1										
NS	0.86	0.85	0.80	−0.30	0.81	0.88	0.91	<u>−0.11</u>	<u>0.07</u>	1.00	<u>0.03</u>	1.00	1.00	<u>−0.06</u>	<u>−0.05</u>	1									
KWI	0.37	0.36	0.37	<u>−0.07</u>	0.76	0.30	<u>0.18</u>	0.54	<u>0.03</u>	0.46	−0.64	0.48	0.47	0.51	−0.38	0.46	1								
KT	0.34	0.25	0.41	0.21	0.19	0.63	0.30	<u>0.16</u>	<u>0.14</u>	0.44	−0.18	0.43	0.45	<u>0.18</u>	<u>−0.01</u>	0.45	0.26	1							
KL	0.82	0.76	0.85	<u>−0.01</u>	0.49	0.67	0.86	−0.43	0.41	0.78	0.39	0.78	0.78	−0.42	<u>0.12</u>	0.78	<u>0.05</u>	0.41	1						
KE	<u>0.17</u>	0.22	<u>0.11</u>	−0.25	<u>0.15</u>	<u>−0.18</u>	0.26	−0.44	<u>0.04</u>	<u>0.08</u>	0.44	<u>0.09</u>	<u>0.08</u>	−0.46	<u>0.10</u>	0.08	−0.20	−0.76	0.22	1					
KDa	0.72	0.64	0.77	<u>0.08</u>	0.62	0.78	0.65	<u>0.07</u>	0.29	0.79	<u>−0.14</u>	0.79	0.79	<u>0.08</u>	<u>−0.09</u>	0.79	0.54	0.83	0.73	−0.38	1				
KDg	0.67	0.59	0.73	<u>0.10</u>	0.57	0.77	0.61	<u>0.09</u>	0.27	0.75	<u>−0.15</u>	0.75	0.75	<u>0.10</u>	<u>−0.08</u>	0.75	0.51	0.88	0.69	−0.45	1.00	1			
KSPH	−0.37	−0.37	−0.34	<u>0.13</u>	<u>−0.02</u>	<u>−0.10</u>	−0.51	0.71	−0.23	−0.25	−0.72	−0.25	−0.24	0.70	−0.26	−0.24	0.51	0.36	−0.62	−0.76	<u>0.09</u>	<u>0.13</u>	1		
KRa	−0.39	−0.35	−0.41	<u>−0.04</u>	<u>0.12</u>	−0.32	−0.55	0.70	−0.29	−0.30	−0.74	−0.29	−0.30	0.68	−0.35	−0.30	0.62	−0.17	−0.75	−0.29	−0.22	−0.22	0.82	1	
KS	0.66	0.58	0.72	<u>0.10</u>	0.57	0.77	0.60	<u>0.10</u>	0.25	0.75	<u>−0.15</u>	0.74	0.75	<u>0.11</u>	<u>−0.07</u>	0.75	0.51	0.88	0.68	−0.45	1.00	1.00	<u>0.14</u>	−0.21	1

Abbreviations used in Table 10 are as follows: KC = Kernel Content; KDa = Kernel Arithmetic Mean Diameter; KDg = Kernel Geometric Diameter; KE = Kernel Elongation; KL = Kernel Length; PK = Percent Kernel; KRa = Kernel Aspect Ratio; KS = Kernel Surface Area; KSPH = Kernel Sphericity; KT = Kernel Thickness; KW = Kernel Weight; KWI = Kernel Width; NDa = Nut Arithmetic Mean Diameter; NDg = Nut Geometric Mean Diameter; NE = Nut Elongation; NL = Nut Length; NRa = Nut Aspect Ratio; NS = Nut Surface Area; NSI = Nut Shape Index; NSPH = Nut Sphericity; NT = Nut Thickness; NV = Nut Volume; NW = Nut Weight; NWI = Nut Width; SW = Shell Weight.

4. Discussion

Artificial pollination has been performed on many fruit species, such as date palm, kiwi fruit, olive, sweet cherry, almond, and pistachio [1,7,41], with variable results. Some studies have been already carried out for hazelnut [12,18,19]. Ellena et al. [19] used a handheld blower and a dry carrier, while [18,33] used a backpack sprayer or mist blower with a liquid carrier, observing an increasing in the fruit set (50%) compared to hand application and an increasing in yield (37%) compared to wind control.

The artificial pollination applied on ‘Tonda Francescana®’ flowers achieved a better result for fruit set (+22.8%) as well as yield (+26.3%), compared to that obtained under ‘open pollination’ by wind when ‘Nocchione’ pollen was used, in agreement with a previous study [12]. The ‘Nocchione’ cultivar has been confirmed as a valid pollinizer for ‘Tonda Francescana®’, while ‘Camponica’ was allowed to obtain a similar fruit set to that obtained with open pollination, as in previous studies [12]. The fruit sets ranged from 2.25 to 2.97 fruits per glomeruli, and this result agreed with that obtained in a previous study on ‘Tonda Francescana®’; it was higher than those values obtained by several authors for other hazelnut cultivars [6,14,42]. A previous study on ‘Tonda Francescana®’ was carried out by applying pollen to the stigma with a paint brush, keeping the flower branches inside a paper bag and, later, keeping nuts in net bags, without evaluation of the effects of ‘manual pollination’ on yield [12]. Artificial pollination by the Pollen Blower machine was applied to 60 plants for each pollinizer, and this allowed us to validate the previous experimental results on the different efficacy of pollinizers thanks to the data of hazelnut yield from plants grown in a commercial hazelnut orchard.

In the hazelnut orchards, the opportunity to improve pollination by artificially spraying compatible pollens that could improve fruit quality requires prior consideration of the xenia effects that the chosen pollinizers might have on nuts and kernels [42].

The xenia effect observed among different cultivars may explain the higher number of blank fruits for ‘Nocchione’. The xenia effect can impact both the endosperm and the embryo formed after double fertilization, in addition to influencing various fruit parameters, such as ripening period, shape, size, color, chemical composition, and nutritional quality [14,43]. The observed effect of ‘Nocchione’ pollen on producing blank nuts agreed with that of another study carried out on ‘Tonda Francescana®’, even if a lower value was observed [12]. The other two pollinizers, ‘Camponica’ and ‘San Giovanni’, had a percentages of undeveloped nuts, respectively, of 7.8% and 9.3%. These ranges are like those observed in other studies [13,14,43]. Beyhan and Marangoz [13] reported that varietal differences in the frequency of blanks as well as important year-to-year variations may be greater than 25% of the crop, and the results of the previous studies varied due to cultivars and ecology.

The effects of the three pollinizers on kernel percentage compared to the control were not significant, in agreement with other studies carried out on hazelnut, almond and pistachios [6,15,23]. Regarding the nut shape index, it was found to be around 0.9, meaning that the ‘Tonda Francescana®’ nuts have a spheroidal shape and NSI was little affected by the three tested pollinizers; in fact, only the pollen of ‘Camponica’ showed slight variations in shape with an increase in SHI. In the literature, the NSI values of different cultivars have been reported as between 0.67 and 1.2, whereas in Tonda Gentile Trilobata was 0.88 and it was stated that this property is quite stable [35].

The ‘Camponica’ pollinizer was associated with bigger nuts and larger kernels, and such results are in line with those of other authors [44] who indicated that pollinizers with large hazelnuts induce larger seeds.

Nut weight (NW) was positively correlated with shell weight (SW) and kernel weight (KW), in agreement with Milosevic and Milosevic [35] and Yao and Mehlen-

bacher (2000) [45]. While kernel weight (KW) was positively correlated with all nut dimensions and kernel diameters, there was no correlation between shell thickness and nut dimensions. This discrepancy between our results and other studies [34,44] is attributable to the fact that the present study evaluates pollinizer-induced xenia on the fruits of a single variety ('Tonda Franciscana®') and not the differences among multiple genotypes.

5. Conclusions

This study showed the validity of artificial pollination on hazelnuts as a tool to ensure fruit set and to achieve higher yield; in fact, with artificial pollination the same results are achieved as with open pollination. For hazelnut, which is a monoecious species with diclinous flower species, asynchrony between staminate and pistillate blossoms is getting more frequent year by year due to climate change [4,5,11], so artificial pollination can reduce reliance on pollinizers as pollination can occur when the principal cultivar is ready [1]. Artificial pollination turned out to be a good tool to increase yield, but only if pollen was used from effective pollinators, and it could be used in unfavorable environmental conditions for pollination, such as frequent rainfall or when there is a lack of pollen. In this study, in fact, artificial pollination showed excellent results only when the pollen of a particularly efficient pollinator, previously assessed as such, for the 'Tonda Franciscana' cultivar was used [12].

Specifically, 'Nocchione' as a pollinizer of 'Tonda Franciscana®' showed better results than 'San Giovanni'.

In terms of xenia effects, 'Nocchione' as a pollinizer of 'Tonda Franciscana®' exhibits a higher number of blank fruits compared to the other pollinizers, without any significant effects on nut and kernel traits, while 'Camponica' was associated with bigger nuts and larger kernels and 'San Giovanni' was associated with a higher nut elongation. Finally, the 'Tonda Franciscana®' shape features were linked mainly to nut weight, which was positively correlated with all nut dimensions and kernel diameters, while no correlation was observed between shell thickness and nut dimensions.

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