

Pollen-Parent Variety Influences Burr Set, Number of Nuts Per Burr, Nut Weight and Shape, and Productivity Index of 'Marrone di Chiusa Pesio'

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ABSTRACT. The 'Marrone di Chiusa Pesio' is among the most well known Italian 'Marrone' varieties. It merits special attention due to its very high nut quality, yet production is notoriously low. Xenia, the direct effect of pollen on the nuts produced has been recognized as a factor in determining the size, time of ripening, peelability and possibly other quality characteristics of chestnuts. Controlled crosses were made in order to identify the best varieties to use as pollinizers for new orchard plantations of 'Marrone di Chiusa Pesio' and to test the reciprocal compatibility among the best of the pollinizer varieties. Fruit set (number of filled burrs per female inflorescence) and number of chestnuts per burr were determined at harvest. Each nut was measured for fresh weight, height, width, thickness and length and width of hylum scar. The number of nuts per kg and the productivity index (number of chestnuts per 100 female inflorescences) were calculated. The Euro-Japanese hybrids 'Bournette,' 'Marsol,' and 'Précoce Migoule,' and the European cultivars 'Belle Epine' and 'Castagna della Madonna' are all capable of effective pollination of 'Marrone di Chiusa Pesio.' The European cultivars appear to be better pollinizers compared to the hybrids. The hybrid pollinizers are reciprocally compatible and 'Précoce Migoule' and 'Belle Epine' are compatible with 'Castagna della Madonna.'

Self incompatibility and male sterility in cultivated chestnut varieties have been identified as sources of potential problems in intensively managed orchard plantations (4, 9, 23). Traditional chestnut orchards have relied on the abundance of pollen-producing (longistaminate) varieties found growing within the orchard and in the adjacent forest of "wild type" trees. Pollinizer choice becomes critically important as the number of varieties necessarily decreases in a modern orchard situation (2, 20). Variety choice for the main crop will almost certainly include the completely male sterile 'Marrone' varieties because of their unmatched nut quality (3, 8, 10, 19, 27). Due to the fact that the longistaminate cultivars have proven to be almost completely self-incompatible (17), at least two reciprocally compatible pollinizers will need to be included in any orchard of male sterile cultivars (23).

The 'Marrone di Chiusa Pesio' is among the most well-known 'Marrone' varieties. It merits special attention due

its very high nut quality, yet production is notoriously low (10, 13, 22, 24, 25, 29). One of the chief causes of the low production no doubt is the rarity of the clone itself, probably no more than 100 mature and bearing trees of 'Marrone di Chiusa Pesio' are known to exist (1) (Dario Adamo, personal communication). The trees of this clone that have been positively identified are usually isolated relicts of the once extensive Marrone orchards on the flat lands at the mouth of the Pesio Valley. It was reasoned that the isolation of the bearing trees, relatively long distances from the "wild type" forests of the mountain slopes and the scarcity of longistaminate cultivars associated with the few remaining 'Marrone' could all be factors contributing to non-optimum pollination. There is currently high market demand for 'Marrone' and thus much interest in planting this cultivar, yet no information has been published on possible pollinizer choices for it.

The aim of the experiment was to screen the best of the currently available longistaminate cultivars in order to identify potential pollinizer varieties for new orchard plantations of 'Marrone di Chiusa Pesio' and to test the reciprocal compatibility among the best of the pollinizer varieties. Xenia, the direct effect of pollen on the fruit or seed, has been recognized as a factor in determining the size, time of ripening, peelability and possibly other quality characteristics of chestnuts (6, 9, 14, 16, 18, 26, 27, 28). It is therefore of considerable importance that the selection of proper pollinizer variety should increase fruit set and productivity but should not decrease nut quality. Additionally, requirements for good pollinizers include contemporaneous blooming with the 'Marrone,' reciprocal intercompatibility and marketable nut quality.

MATERIALS AND METHODS

A series of Euro-Japanese hybrids, whose reciprocal fertility was currently under investigation (17) was reviewed for use in the pollination study. Hybrids were chosen because of their potential value to a modern chestnut orchard system, being large fruited, relatively very precocious and early ripening and resistant to *Cryphonectria parasitica* (Murr.) Barr and *Phytophthora* spp. (12, 21). Cultivars of *Castanea sativa* Mill. from the provinces of Cuneo and Torino also were reviewed. Only one longistaminate variety, 'Castagna della Madonna' grown in the Roero, had the requisite quality characteristics to merit trial; the nut

ripens very early, is fairly large, and commands a high price and a fair share of the early-season market (13). 'Belle Epine,' a French longistaminate variety of excellent quality (15), was included on the basis of its performance in Piemonte (21).

The research was carried out during 1989-1990 at Chiusa Pesio (CN) and in 1990 at Montaldo Roero (CN). The trees chosen for the pollination at Chiusa Pesio were adult clones of 'Marrone di Chiusa Pesio' grown in a level irrigated site at 575 m altitude. The tree chosen at Montaldo Roero was an adult clone of 'Castagna della Madonna.' At Chiusa Pesio, controlled pollinations were made with freshly collected pollen of the hybrid cultivars 'Bournette,' 'Marsol' and 'Precoce Migoule' and the European cultivars 'Belle Epine' and 'Castagna della Madonna.' Controlled pollinations also were made with "wild type" pollen collected from the adjacent forest. At Montaldo Roero controlled pollinations were made with freshly collected pollen of 'Belle Epine,' 'Précoce Migoule' and, for the self-compatibility test, 'Castagna della Madonna.' Unfortunately, the 'Belle Epine' and 'Précoce Migoule' trees were too small to provide adequate pollen for the study at Chiusa Pesio and be used as seed parents in reciprocal pollinations with 'Castagna della Madonna.'

Approximately 2 wk before the beginning of anthesis, which occurred between the end of June and the beginning of July for each pollen type and for the open pollinations, 12 flowering branches at Chiusa Pesio and 5 at Montaldo Roero were selected. Each selection had as uniform as possible growth habit, exposure and number of bisexual catkins. Chosen branches were isolated with paper bags to exclude all airborne pollen and labelled with the number of female inflorescences present on the branch. At the time of bagging in Montaldo Roero, all male catkins and the male portion of the bisexual catkins were removed from the selected branches. Branches of the chosen pollinizer varieties were isolated with paper bags in order to obtain male catkins not contaminated with other pollen.

In 1989, 12 branches also were isolated to test the possibility of self-fertility and apomixis of 'Marrone di Chiusa Pesio.' Based on the results (total absence of self-fruitfulness), it was decided not to repeat this test the following year. The bags were opened at the moment of full anthesis and the pollination was effectuated by brushing the male catkins across the female inflorescence. Pollen collection and handling was limited to simply removing the portion of the bagged branch with the bag still attached for transportation to the pollination site. Male catkins were removed from the bags as needed. The pollinations were repeated after one week.

About 20 days later, when danger of open cross-pollination had passed, the paper bags were removed and substituted with large mesh bags to improve aeration and to recover every burr and chestnut produced by each labelled branch.

Fruit set and number of chestnuts per burr were determined at harvest, which occurred toward the end of September for 'Castagna della Madonna' and in mid-October

for 'Marrone di Chiusa Pesio.' Each nut was measured for fresh weight, height, width, thickness and length and width of the hylum scar. The number of chestnuts per kilogram and the productivity index (number of chestnuts per 100 female inflorescences) were calculated. The productivity index, derived from the product of the number of chestnuts per burr and the number of burrs set, allows a better evaluation of the fecundity of the pollinizers. All data were subjected to statistical analyses after transformation into angular values by the method of Bliss for a one-way analysis of variance (7). Tukey's range test was used for significant differences at 95 and 99 percent levels. The data from the open pollinations, although included in the tables, were not included in the statistical analysis. The isolation of the female flowers by bagging and manipulation of the pollen for the cross pollinations constitute variables that preclude comparison with the open pollinations.

RESULTS

The 1989 data from Chiusa Pesio show, above all, that pollen of 'Castagna della Madonna' had a significantly greater fecundity as measured by the burr-set with respect to the other pollinizers (Table 1). Also, the number of chestnuts per burr tended to be higher for 'Castagna della Madonna,' although statistically significant only when compared to 'Marsol' and "wild type."

The productivity index underlines the superiority of the 'Castagna della Madonna' pollen when compared to the other pollinizer varieties, even though significantly different only from "wild type." Furthermore, chestnuts derived from pollination by 'Castagna della Madonna' were found to be larger in every dimension with respect to fruit of the other pollinations, with highly significant differences for height, width, and average weight (Table 2). Also, the hylum scar was found to be significantly larger for seeds whose pollen parent was 'Castagna della Madonna' (Table 3).

Results for the 1990 season at Chiusa Pesio (Table 4) show that, in general, fruit set was lower than in the previous year. 'Belle Epine' and "wild type" pollens gave the highest fruit set and were significantly higher than 'Marsol.' There were no statistical differences among pollens for number of chestnuts per burr. Table 4 also shows that the productivity index was highest for 'Belle Epine' and was significantly higher than 'Précoce Migoule' and 'Marsol.'

Table 5 shows the means for fruit size and number of chestnuts per kilogram; it indicates that 'Castagna della Madonna' pollen had a less pronounced positive influence on size and weight of the nuts than in the previous year. 'Belle Epine' pollen, however, tended to produce larger and heavier nuts. Differences in the size of the hylum scar also were less pronounced than in the previous year (Table 6).

Pollen parent influenced the shape of the chestnuts as well, and is shown by the ratio of height to width (Table 7). The most oblong chestnuts were produced by 'Castagna della Madonna' pollen for both years. 'Bournette' pollen produced "rounder" chestnuts than most of the other

Table 1. Results of the 1989 pollination on 'Marrone di Chiusa Pesio.' Percent fruit set, chestnuts per burr and productivity index.*

| Pollinizer | Fruit Set (%) | Chestnuts Per Burr | Productivity Index |
|------------------------|---------------|--------------------|--------------------|
| "Wild type" | 48.9 b | 1.49 c | 72.9 Bb |
| Bournette | 64.7 b | 1.95 ab | 126.2 ABab |
| Marsol | 63.1 b | 1.53 b | 96.5 ABb |
| Précoce Migoule | 65.8 b | 1.84 abc | 121.1 ABb |
| Castagna della Madonna | 87.2 a | 2.02 a | 176.1 Aa |
| Open | 47.4 | 1.57 | 74.4 |

Table 2. Results of the 1989 pollination on 'Marrone di Chiusa Pesio.' Nut dimensions and number of chestnuts per kilogram.*

| Pollinizer | Height (mm) | Width (mm) | Thickness (mm) | Nuts/kg |
|-------------|-------------|------------|----------------|----------|
| "Wild type" | 29.5 BCb | 32.0 BCcd | 21.4 ABab | 87.18 Bb |
| Bournette | 28.7 Cc | 31.7 Cd | 19.8 Bc | 95.73 Aa |
| Marsol | 29.0 BCbc | 33.1 BCbc | 22.1 Aab | 81.70 Bb |
| P. Migoule | 29.9 Bb | 33.8 Bb | 21.0 ABb | 81.57 Bb |
| C. Madonna | 31.2 Aa | 36.9 Aa | 22.3 Aa | 64.77 Cc |
| Open | 28.1 | 34.0 | 22.0 | 78.24 |

Table 3. Results from 1989 pollination on 'Marrone di Chiusa Pesio.' Dimensions of the hylum scar.*

| Pollinizer | Width (mm) | Thickness (mm) |
|------------------------|------------|----------------|
| "Wild type" | 20.9 BCbc | 13.0 Bb |
| Bournette | 20.3 Cc | 12.1 Bc |
| Marsol | 20.1 Cc | 12.4 Bbc |
| Précoce Migoule | 21.5 Bbc | 12.6 Bbc |
| Castagna della Madonna | 24.7 Aa | 14.2 Aa |
| Open | 21.8 | 12.9 |

Table 4. Results of the 1990 pollination on 'Marrone di Chiusa Pesio.' Percent fruit set, chestnuts per burr and productivity index.*

| Pollinizer | Fruit Set (%) | Chestnuts Per Burr | Productivity Index |
|------------------------|---------------|--------------------|--------------------|
| "Wild type" | 70.5 a | 1.74 n.s. | 122.7 ab |
| Bournette | 51.7 ab | 1.96 n.s. | 101.3 ab |
| Marsol | 33.4 b | 1.73 n.s. | 57.8 b |
| Précoce Migoule | 42.0 ab | 1.57 n.s. | 65.9 b |
| Castagna della Madonna | 60.2 ab | 1.70 n.s. | 102.3 ab |
| Belle Epine | 73.4 a | 2.04 n.s. | 149.7 a |
| Open | 48.4 | 1.56 | 75.5 |

Table 5. Results of the 1990 pollination trial on 'Marrone di Chiusa Pesio.' Nut dimensions and number of chestnuts per kilogram.*

| Pollinizer | Height (mm) | Width (mm) | Thickness (mm) | Nuts/kg |
|-------------|-------------|------------|----------------|-------------|
| "Wild type" | 28.2 Cc | 36.0 b | 21.9 ab | 73.31 ABab |
| Bournette | 29.4 ABa | 36.5 ab | 21.5 Aa | 75.10 Aa |
| Marsol | 28.9 ABCabc | 36.1 ab | 22.9 ab | 69.11 ABabc |
| P. Migoule | 29.4 ABCab | 37.1 ab | 23.5 a | 66.98 ABbc |
| C. Madonna | 28.5 BCbc | 36.7 ab | 23.0 | 68.63 ABabc |
| Belle Epine | 29.6 Aa | 37.4 a | 22.6 ab | 66.31 Bc |
| Open | 30.1 | 36.1 | 23.3 | 71.94 |

Table 6. Results from 1990 pollination on 'Marrone di Chiusa Pesio.' Dimensions of the hylum scar.*

| Pollinizer | Width (mm) | Thickness (mm) |
|------------------------|------------|----------------|
| "Wild type" | 22.9 b | 12.8 b |
| Bournette | 23.0 b | 13.2 ab |
| Marsol | 23.1 b | 13.4 ab |
| Précoce Migoule | 23.8 ab | 13.0 ab |
| Castagna della Madonna | 22.8 b | 13.3 ab |
| Belle Epine | 24.4 a | 13.6 a |
| Open | 23.1 | 13.1 |

Table 7. Nut shape as expressed by the ratio height:width for both years' pollinations on 'Marrone di Chiusa Pesio.'*

| Pollinizer | 1989 | 1990 |
|------------------------|---------|-----------|
| "Wild type" | 0.97 Aa | 0.78 n.s. |
| Bournette | 0.91 Bb | 0.81 n.s. |
| Marsol | 0.88 Bc | 0.80 n.s. |
| Précoce Migoule | 0.88 Bc | 0.79 n.s. |
| Castagna della Madonna | 0.84 Cd | 0.78 n.s. |
| Belle Epine | | 0.79 n.s. |
| Open | 0.83 | 0.84 |

Table 8. Results of the 1990 pollination on 'Castagna della Madonna.' Percent fruit set, chestnuts per burr and productivity index.*

| Pollinizer | Fruit Set (%) | Chestnuts Per Burr | Productivity Index |
|-----------------|---------------|--------------------|--------------------|
| Précoce Migoule | 50.9 n.s. | 1.88 Bb | 88.4 Bb |
| Belle Epine | 58.6 n.s. | 2.59 Aa | 146.2 Aa |
| Open | 38.9 | 0.6 | 25.0 |

Table 9. Results of the pollination trial on 'Castagna della Madonna.' Dimensions of the nuts and number of chestnuts per kilogram.*

| Pollinizer | Height (mm) | Width (mm) | Thickness (mm) | Nuts/kg | H:W |
|-------------|-------------|------------|----------------|---------|------|
| P. Migoule | 28.49 | 32.59 | 20.78 | 97.4 | 0.88 |
| | n.s. | Cc | Bb | Bb | Aa |
| Belle Epine | 28.15 | 33.36 | 19.68 | 108.7 | 0.85 |
| | n.s. | Bb | Cc | Aa | Bb |
| Open | 28.15 | 35.25 | 24.15 | 80.18 | 0.80 |

Table 10. Results from pollinations on 'Castagna della Madonna' showing dimensions of the hylum scar.*

| Pollinizer | Width (mm) | Thickness (mm) |
|-----------------|------------|----------------|
| Précoce Migoule | 25.0 Bb | 14.9 n.s. |
| Belle Epine | 26.5 Aa | 14.8 n.s. |
| Open | 27.8 | 15.95 |

*Means separation in columns with Tukey's range test, $P \leq 0.05$ (lowercase letters), $P \leq 0.01$ (uppercase letters).

pollen varieties in both years. It should be mentioned that the "characteristic" desired shape of the 'Marrone' is decidedly oblong (10, 11).

The results from the pollinations at Montaldo Roero on the 'Castagna della Madonna' show that both pollinizers were compatible with 'Castagna della Madonna.' There were no differences in fruit set, but the number of nuts per burr was significantly higher when the pollen parent was 'Belle Epine' (Table 8). Consequently, the resulting chestnuts were thinner on the average since the central nut, when there were three per burr, was flattened on both sides (Table 9). Measurements of the hylum are given in Table 10.

DISCUSSION

The results of this experiment should not be considered conclusive, nor should they be generalized for situations other than those in which these observations were made. Only two years of data are represented by this work and relatively very few potential pollinizer cultivars have been investigated. It may be said, however, that the controlled pollinations with the pollinizers examined have shown, for both years, a fecundity at least equal to and often greater than open pollination. It should be pointed out that overall, the European cultivars appear to be better pollinizers than the Euro-Japanese hybrids for 'Marrone di Chiusa Pesio.' Both 'Belle Epine' and 'Précoce Migoule' are suitable pollinizers for 'Castagna della Madonna.' However, the results of this experiment should not be considered conclusive as only two years of data are represented and relatively very few potential pollinizer cultivars were investigated.

The most surprising and possibly most important result shows the pollen effect on the nut quality characteristics of the 'Marrone.' The nuts resulting from pollination by the European pollinizer varieties were larger and more oblong-shaped for both years of study, when compared to the hybrids. This production of larger nuts was observed despite higher fruit set and a higher number of chestnuts per burr. These data appear to confirm the previous reports of the xenia phenomenon, as mentioned in the introduction.

Quality parameters for 'Marrone' varieties include size, shape, wholeness of kernel (not divided into multiple seeds), peelability, and texture after cooking, among others (11). If these are all xenia influenced characters, the problem deserves serious attention and should be verified by experiments specifically designed to test the hypothesis. The work represented by the data presented here was designed to test pollinizer compatibility in a more general way and not specifically for xenia effects, and thus, did not control for some rather important variables. Variables not controlled include choice of "wild type" material that was casually selected from a tree at the border of the forest rather than from a specific tree and was different for the two years. A better control would be a mix of "wild type" pollens gathered from more than one individual in a representative population.

Multiple-seeded nuts, a serious quality defect, is thought to be genetically controlled and is one of the characters that should be used to distinguish 'Marrone'

from ordinary chestnuts (4). The pollen effect on the number of embryos has never been determined and since the seeds from this study were saved for planting, this parameter was not investigated. The interaction of year and phenotype was not investigated, but Bignami and Mastrantonio (5) showed very strong significance for the factor "year" for all fruit parameters studied in a population of 'Marrone di Viterbo.' In addition, they noted a strong interaction "year x plant" within already significant "locality" differences for plants of the same clone. Although not mentioned by the authors, it appears that pollination differences and xenia effects may have contributed to the variability that they observed.

In summary, Euro-Japanese hybrid cultivars 'Bournette,' 'Marsol,' and 'Précoce Migoule,' and the European cultivars 'Belle Epine' and 'Castagna della Madonna' are all capable of effective pollination and fertilization of the highly valued 'Marrone di Chiusa Pesio.' The European cultivars appear to be better pollinizers compared to the hybrids when measured parameters include fruit set, number of chestnuts per burr, size, shape and weight of the chestnuts, and the overall productivity index. The hybrid pollinizers are reciprocally compatible and 'Précoce Migoule' and 'Belle Epine' are compatible with 'Castagna della Madonna.' The model orchard would include a 'Marrone' and a minimum of two reciprocally compatible pollinizers for which the xenia phenomena has been determined to have no adverse and possibly to have a positive effect on the 'Marrone' nut quality.

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