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Pomology / Original Article

Manual pollination of non-self-pollinating Arbequina olive trees using three pollen-donor cultivars

Abstract – The objective of this work was to evaluate how the productivity of the non-self-pollinating olive cultivar Arbequina is affected by manual pollination with pollen from cultivars Coratina, Pendolino, and Koroneiki in Brazil. The study was conducted over two crop years, and the donor cultivars were chosen due the proximity of their flowering periods. The inflorescences were bagged during the pre-flowering stage, and an in vitro germination test was subsequently conducted to verify the viability of the pollen grains. The first pollen application occurred on the third day after the anthesis of the donor cultivars, and the second, seven days later. Afterwards, production analyses were conducted. In both study years, 'Coratina' stood out as a pollen donor compared with 'Arbequina' (self-pollinated control) regarding the parameters number of viable fruits (2.38 and 4.30% vs. 0.5 and 0.42%), production per plant (23.05 and 41.64 kg per plant vs. 4.84 and 4.06 kg per plant), and productivity (6,569.25 and 11,868.54 kg ha^{-1} vs. 1,379.97 and 1,159.09 kg ha^{-1}). The pollination of 'Arbequina' with pollen from 'Coratina' increases the number of viable fruits and, consequently, favors crop productivity.

Index terms: *Olea europaea*, Coratina, phonology, physiology.

Polinização manual de oliveiras Arbequina não autopolinizantes com uso de três cultivares doadoras de pólen

Resumo – O objetivo deste trabalho foi avaliar como a produtividade da cultivar de oliveira Arbequina, não autopolinizante, é afetada pela polinização manual com pólen das cultivares Coratina, Pendolino e Koroneiki no Brasil. O estudo foi conduzido durante dois anos-safra, e as cultivares doadoras foram escolhidas devido à proximidade dos seus períodos de floração. As inflorescências foram ensacadas durante a fase de pré-floração, e um teste de germinação in vitro foi realizado posteriormente para verificar a viabilidade dos grãos de pólen. A primeira aplicação de pólen ocorreu no terceiro dia após a antese das cultivares doadoras, e a segunda, sete dias depois. Em seguida, foram realizadas análises de produção. Nos dois anos de estudo, 'Coratina' destacou-se como doadora de polén em comparação à 'Arbequina' (controle autopolinizado) quanto aos parâmetros número de frutos viáveis (2,38 e 4,30% vs. 0,5 e 0,42%), produção por planta (23,05 e 41,64 kg por planta vs. 4,84 e 4,06 kg por planta) e produtividade (6.569,25 e 11.868,54 kg ha^{-1} vs. 1.379,97 e 1.159,09 kg ha^{-1}). A polinização da 'Arbequina' com pólen da 'Coratina' aumenta o número de frutos viáveis e, consequentemente, favorece a produtividade da cultura.

Termos para indexação: *Olea europaea*, Coratina, fenologia, fisiologia.

Introduction

The cultivation of the olive (*Olea europaea* L.) tree spans millennia, making it one of the oldest crop on record (Zhu et al., 2013). This fruit-bearing tree, belonging to the Oleaceae family, produces fruit called a drupe, commonly known as an olive, and originated from the region stretching from the southern Caucasus to the plains of Iran, across all countries bordering the Mediterranean, with the most significant traces found in Syria (Sánchez-Estrada & Cuevas, 2018).

Due to the steady increase in the consumption of functional and healthy foods, there has been a growing demand for olive oil in Brazil, which currently imports 99.6% of the product consumed in the country (Witter et al., 2019). However, in the state of Rio Grande do Sul, a significant increase of 202 Mg L^{-1} in olive oil production was observed in the last harvest of 2022, representing approximately 120 and 311% more than in the 2021 and 2020 harvests, respectively (Ambrosini et al., 2022). This result is attributed to a substantial increase in the planted area, as well as to the orchards entering the production phase (Ambrosini et al., 2022), which occurs biennially. Biton et al. (2020) highlighted that the olive tree is considered alternate bearing, taking two years to complete its production cycle, which means that two reproductive cycles occur simultaneously, leading to a competition phenomenon for products generated during photosynthesis.

For olive cultivation, it is essential to understand the floral biology and genetic compatibility of the olive tree (Mazzeo et al., 2014), whose pollination can occur through self- or cross-pollination. However, cross-pollination can lead to a more significant production even in varieties considered self-fertile, remaining critic for certain species that have anomalies in their reproductive organs (Sánchez-Estrada & Cuevas, 2020). Pinillos & Cuevas (2009), for example, found that 'Picual' olive trees are self-incompatible, showing an improved production when artificially cross-pollinated with donor material from 'Hojiblanca'. Moreover, considering self- and inter-incompatibility issues between some varieties, coinciding flowering periods do not necessarily guarantee an effective fruit set, indicating that knowledge of which cultivars can self-pollinate and genetically match is vital (Marchese et al., 2016).

According to the literature, multiple factors influence pollination and subsequent fruit production, such as floral phenology (Witter et al., 2019) and the interplay between gibberellins synthesized in the fruit seeds, which inhibits auxins in the growth tips of the plant, partially affecting floral induction (Riella et al., 2022). The natural pollination process is further complicated by the highly-complex floral anatomy of olive trees, as well as by their flowering period.

In the state of Rio Grande do Sul, the flowering period is short, occurring in September and October (Mazzeo et al., 2014). According to these same authors, flowers should undergo pollination and fertilization for fruit formation and development, processes in which relative humidity plays a critical role: when below 50%, it reduces stigma viability, but, when close to 100%, it causes pollen to undergo hydration and gain weight, diminishing the efficacy of anemophilous pollination (Mazzeo et al., 2014). Moreover, excessive hydration can result in an undesired increase in size, potentially leading to the destruction of the pollen (Biton et al., 2020). In cases where complete or double fertilization fails to occur, there is the production of parthenocarpic fruits, which are smaller than the normally fertilized counterparts, lacking economic value and often remaining on the tree until harvest (Sánchez-Estrada & Cuevas, 2020).

In Brazil, the Arbequina olive cultivar is one of the most cultivated. In the county, a drop in the production of Arbequina has been observed in orchards without the presence of other cultivars to pollinate it, such as the recommended 'Koroneiki', contrasting with its behavior in its region of origin in Spain, where it is considered self-pollinating (Marchese et al., 2016). However, in the field, there is an abundant flowering of both cultivars and coincident flowering periods, but a satisfactory fixation only in 'Koroneiki', a phenomenon also reported in the Italian region of Sicily by those same authors. Cultivar Arbequina may also be pollinated by Arbosana, although both appear to present self and inter-incompatibility issues (Centeno et al., 2019), i.e., these cultivars self-pollinate through cross pollen but are also incompatible with pollen from some other cultivars.

The objective of this work was to evaluate how the productivity of the non-self-pollinating olive cultivar Arbequina is affected by manual pollination with pollen from cultivars Coratina, Pendolino, and Koroneiki in Brazil.

Materials and Methods

The experiment was carried out over two crop years, from August 2021 to the end of March 2022, and repeated in the same months in 2022–2023. The experimental area, with a northeast-southwest solar exposure, was located on the premises of the Rigo Vinhedos e Olivais company, in the municipality of Dom Pedrito, in the state of Rio Grande do Sul, Brazil (31°08'41.72"S, 54°11'43.41"W, at 350 m of altitude). According to Köppen's classification, the climate of the region is of the Cfa type, humid subtropical, with rainfall well distributed throughout the year. During the experimental period, meteorological data were collected from a weather station located on the property where the research was conducted (Figure 1).

The soil in the area can be characterized as an Argissolo with moderate drainage (Santos et al., 2023), which is analogous to an Ultisol. Soil chemical properties at the beginning of the experiment were: pH (in $CaCl_2$) 5.5, 3.5 $cmol_c$ dm^{-3} Ca, 1.7 $cmol_c$ dm^{-3} Mg, 0.00 $cmol_c$ dm^{-3} Al, 156 $cmol_c$ dm^{-3} K, 6.3 mg dm^{-3} P (Mehlich^{-1}), 3.5 $cmol_c$ dm^{-3} Al+H, and base saturation of 62%. Soil acidity was corrected with the application and incorporation of dolomitic limestone at a rate of 0.75 g dm^{-3} according to the recommendation for the region (Figueiredo et al., 2022).

The study area was selected by leaving both a border row of plants and peripheral plants within the plot to avoid influence from other areas. The olive grove spacing was 5.0 m between plants and 7.0 m between rows, resulting in 285 plants per hectare.

The used cultivar was Arbequina, obtained from commercial areas planted in 2010 and 2012. The used pollen-donor materials were from cultivars Coratina, Pendolino, and Koroneiki, extracted from different cultivation areas within the same property.

The experiment was carried out in a randomized complete block design, with four treatments ('Arbequina' manually pollinated with pollen from 'Coratina', 'Pendolino', and 'Koroneiki', as well as self-pollinated, which used as the control) and five replicates of two plants, totaling 40 sample units.

The control did not involve the application of pollen, only the prior bagging of 'Arbequina' inflorescences. For the other treatments, the pollen from each pollen-donor cultivar was applied twice to Arbequina (Figure 2), as follows: at full flowering on 10/04/2021 and 10/02/2022 and seven days later on 10/11/2021 and 10/09/2022 for 'Coratina', at flowering above 75% on 10/11/2021 and 10/07/2022 and at the end of flowering on 10/18/2021 and 10/11/2022 for 'Pendolino', and on 09/30/2021 and 09/21/2022 and seven days later during full flowering for 'Koroneiki'.

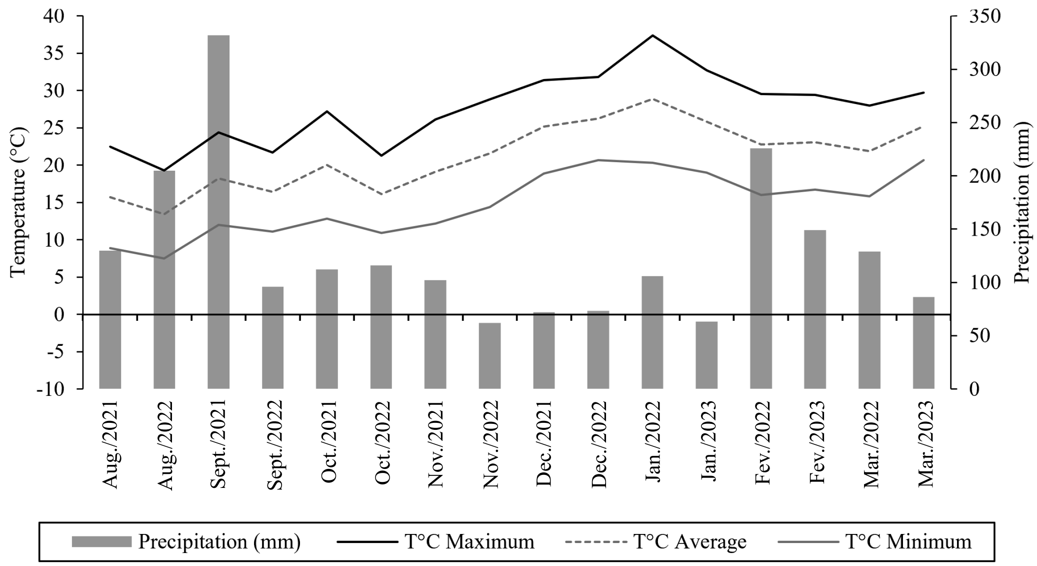

Figure 1. Meteorological data during the experimental years of 2021–2022 and 2022–2023. Data was collected from a meteorological station located at the Rigo Vinhedos e Olivais property, in the municipality of Dom Pedrito, in the state of Rio Grande do Sul, Brazil.

The method used for manual pollen application and evaluation was adapted from Pinillos & Cuevas (2009). Initially, some branches were thinned, and, then, ten remaining branches, each with 12 panicles, were marked, totaling 120 floral racemes per treatment.

During pre-flowering, the inflorescences were covered with transparent plastic bags (35×20 cm, 0.12 µm thick) to prevent unwanted pollination, totaling five buds with 12 panicles on the east side and five on the west side of each sample unit. The pollen was collected during the dehiscence periods of the pollinating cultivars and not of the receiving cultivar Arbequina.

For the first pollination, the plastic bags were removed for manual pollination with each donor and then replaced again. Specifically, the flowers from the branches of the pollinating cultivar were removed three days after anthesis and tapped onto a transparent polyvinyl chloride plastic sheet to obtain the pollen for each treatment. The pollen grains were then placed in a plastic container, and pollen exchange between cultivars was immediately performed using a cotton swab. This process was repeated the following week. Afterwards, the branches were covered with plastic bags again until the second application. Finally, the bags were removed from the inflorescences at the end of the 'Arbequina' flowering period, and the area was revisited only during fruit count.

To determine which treatment had a better effect on improving the overall productivity of the olive grove, the evaluated parameters were: pollen grain viability test (%), effective fruit set (%), total fruit mass (kilogram per plant), average fruit mass (g), and oil yield (%).

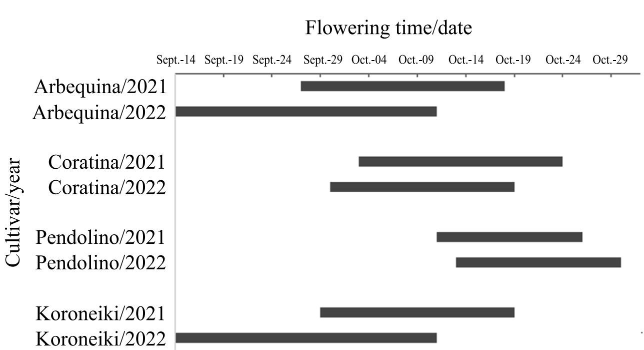

Figure 2. Phenology of the flowers of the Arbequina, Coratina, Pendolino, and Koroneiki olive (*Olea europaea*) tree cultivars used for manual pollination of Arbequina over two years in the municipality of Dom Pedrito, in the state of Rio Grande do Sul, Brazil.

The pollen grain viability test was performed using a method adapted from Silva et al. (2016). A culture medium was prepared by dissolving sugar and agar in warm water in an Erlenmeyer flask. After adding 15% sucrose and 1% agar to 100 mL distilled water, the solution was used to disperse pollen grains onto Petri dishes, which were covered with two sheets of absorbent paper previously moistened with distilled water, simulating a humid chamber. These dishes were then placed in an incubator at a controlled temperature of around 24°C for 48 hours. Germinated and non-germinated pollen grains were observed at random and manually counted until reaching 100 using a tabletop microscope with a 10× eyepiece and 10× objective lens. The pollen grains with a pollen tube length equal to or greater than the diameter of the pollen itself were considered viable (Coutinho et al., 2009).

The effective fruit set was obtained by counting the viable number of fruits 45 days after anthesis. An average of 18 inflorescences per panicle was used for the Arbequina cultivar, with 120 panicles considered per sample unit, aiming to identify which treatment had the most significant impact on the productivity of the olive grove.

Total fruit mass was sampled from 100 fruits collected randomly at harvest from each treatment. For each treatment, fruit mass per plant and, consequently, productivity per hectare were measured in kg ha^{-1}, and total mass per experimental unit was determined to be linked to final production. Average fruit mass was obtained by weighting 100 fruits, also collected randomly.

To obtain olive oil yield, 5.0 kg fruit samples from each treatment were processed at the Olive Oil Laboratory of Embrapa Clima Temperado using the Abencor system (MC2 Ingerniería y Sistemas, S.L., Sevilla, Spain). The steps carried out were: fruit rupture in the MM-100 hammer mill, dough thermal-beating phase in the TB 100 thermo-mixer, and centrifugation in the CF100 centrifuge (MC2, Ingerniería y Sistemas, Seville, Spain). Then, the samples were placed in a beaker for decantation, and the oil was finally removed from the container without moving the sludge deposited at the bottom, generating the average oil yield. The final olive oil yield for each treatment was used to determine the increase in liters per hectare.

The data obtained for the other variables were subjected to the analysis of variance. Normality and homoscedasticity were determined through the tests of Shapiro-Wilk and Hartley, respectively. Means were compared by Tukey's test ($p<0.05$) using the WinStat statistical software (Machado & Conceição, 2002).

Results and Discussion

Among the variables analyzed for pollen grain viability, no statistical differences were observed between the donor cultivars. The means obtained in 2022 and 2023 were 44.95% and 53.57%, respectively, which is an indicative that the inefficiency of some donor cultivars was not due to their lower fertility (Table 1). Similar pollen viability results were reported by Coutinho et al. (2009).

When evaluating the effects of different environmental conditions, Iovane et al. (2022) found that temperatures above 30ºC and relative air humidity close to 100% were harmful to the germination of olive pollen grains. In the present study, temperatures were below 30ºC during the viability analysis. In 2021, relative humidity reached close to 100% in the mornings throughout the pollen development stage, which resulted in a decrease in pollen viability since, in wind-dependent cross-pollination, the higher the relative humidity, the lower the number of pollen grains that are detached from the anthers (Figure 1).

According to Rallo-Romero & Cuevas (2017), olive tree genotypes require suitable climatic conditions for floral development, which conditions the amount of viable gametes during anthesis (Coutinho et al., 2009). In the present work, the climate in the experimental region did not play a determining role in hormonal balance and, consequently, in the development of gametes. However, the increase in rainfall in the month before flowering, when part of the floral organs were maturing, increased the probability of a reduction in viable gametes throughout anthesis (Coutinho et al., 2009).

The rainfall volumes in the experimental years partially influenced pollen viability. From August 2021 to March 2022 and from August 2022 to March 2023, rainfall stability was observed (Figure 1), with an average of 142 mm per month. In February 2022, August 2022, and September 2021, rainfall exceeded 200 mm, but mainly in the pre-flowering stage. In November 2022, December 2022, January 2023, and December 2021, drought was recorded, with average incidence rates below 60 mm. Under these climate conditions, the pollen viability of all cultivars was lower than that of 64% obtained for 'Arbequina' pollinated with 'Sikitita' by Cuevas et al. (2024). However, this result did not reduce productivity since the olive tree blooms for more than 25 days. Therefore, it is assumed that some of reproductive organs matured under more favorable climatic conditions (Maniriho, 2022).

The maximum, average, and minimum temperatures recorded were 27.2, 18.7, and 10.7°C, respectively (Figure 1). Maximum temperatures, close to 30°C, were observed in January 2022, December 2022, and January 2023, and minimum temperatures, below 9°C, in August 2021 and August 2022 during the dormancy period of the crop, not affecting the quality of the plants.

Regarding effective fruit set, the highest rates of over 2.3 and 4.3% in the first and second year, respectively, were obtained for Coratina, meaning it was the most effective cultivar to pollinate Arbequina (Table 1). These results were consistent across blocks of the experiment and, therefore, are considered reliable. Studies of this nature are important since olive cultivars present different degrees of self- and inter-incompatibility (Castric et al., 2024), which are influenced by the environmental conditions of the planting site. Although the phenology of the Coratina cultivar did not overlap perfectly with that of Arbequina (Figure 2), an average rate of more than 2.0% was still recorded, which is considered excellent for the crop according to Orlandi et al. (2020).

For the variable production per plant, cultivar Arbequina showed better values when pollinated with Coratina, reaching 23.05 and 41.64 kg per plant in the 2022 and 2023 harvests, respectively (Table 1). A similar result was observed for productivity per hectare, which was 6.615 Mg ha^{-1} in the first year and 11.95 Mg ha^{-1} in the second, representing increases of 4.41 and 11.2 times compared to the values obtained with 'Pendolino' and of 3.6 and 10.3 times with 'Koroneiki'.

Although Pendolino is recognized as a good universal pollen donor (Pinillos & Cuevas, 2009), it did not pollinate Arbequina in the present study. This result can be attributed to the short period of overlap between both cultivars since the anthesis of Pendolino occurred when more than 96% of the flowering of Arbequina was already underway (Figure 2). Zhang et al. (2022) obtained more than 100% better results compared to the control treatment (self-pollination) when manually pollinating peony (*Paeonia ostii* T.Hong & J.X.Zhang), which is totally dependent on cross-pollination like the olive tree, reinforcing the importance of investigating this crop.

The Koroneiki cultivar was not able to adequately pollinate Arbequina. In the first year, the increase in fruit production variables was insignificant, whereas, in the second, pollination with 'Koroneiki' was lower than that of the control treatment with self-pollinated 'Arbequina' (Table 1). Furthermore, inter-incompatibility prevented fertilization among these cultivars although their period of stability coincided almost 100%, which makes Koroneiki, the most planted cultivar in Brazil, a viable candidate for the pollination of Arbequina, especially due to its high degree of sporophytic self-incompatibility. Through paternity testing, Marchese et al. (2016) concluded that the few fruits borne by 'Arbequina' resulted from self-pollination rather than from cross-pollination with 'Koroneiki'.

The variable mass of 100 fruits did not show a significant variation between the study years regardless of the treatment (pollen-donating cultivars), with averages of 182.15 g in 2022 and 174 g in 2023 (Table 1). Even though it was not significant, the decrease of almost 10 g, on average, in the mass of 100 fruits between the years is attributed to the climate and the increase in crop productivity in 2023.

The 'Coratina' cultivar stood out for its potential of pollinating 'Arbequina'. A reduction in the mass of 100 fruits was expected, which, however, did not occur, suggesting the possibility of the cultivar having a good nutritional level to the point of not reducing the reserves of the constituent organs. This result is in line with that of the work carried out by Juhász et al. (2009), who found that manual pollination in physic nut (*Jatropha curcas* L.) cultivation resulted in fruits with an equivalent weight, but in a significantly higher number.

The olive oil yield in the samples of the evaluated olive tree cultivars did not differ significantly between both study years, being 13.5% in 2022 and 12.5% in 2023, representing a high number of fruits per plant, which can be attributed to the greater photosynthetic capacity of the 'Arbequina' cultivar. According to Hernandez-Santana et al. (2018), although the needs of each cultivar vary, it is estimated that, on average, around 120 olive leaves are needed to support the development of a single fruit.

Table 1. Pollen grain viability and fruit production variables of Arbequina olive (*Olea europaea*) trees manually pollinated with pollen from donor cultivars (treatment) over two crop years[(1)].

Treatment	Year	Pollen grain viability (%)	Fruit production variables				
			Effective fruit set (%)	Production (kg per plant)	Productivity (kg ha^{-1})	Average mass of 100 fruits (g)	Oil yield (%)
'Arbequina'[(2)]	2022	43.6^{ns}	0.50b*	4.842b*	1,379.970b*	182.5^{ns}	13.5^{ns}
	2023	52.6^{ns}	0.42b	4.067b	1,159.095b	172.5^{ns}	12.5^{ns}
'Coratina'	2022	45.6^{ns}	2.38a	23.050a	6,569.250a	180.7^{ns}	13.5^{ns}
	2023	54.1^{ns}	4.30a	41.644a	11,868.540a	175.7^{ns}	12.5^{ns}
'Pendolino'	2022	45.2^{ns}	0.44b	4.261b	1,214.385b	183.9^{ns}	13.5^{ns}
	2023	53.6^{ns}	0.35b	3.389b	965.865b	173.3^{ns}	12.5^{ns}
'Koroneiki'	2022	45.4ns	0.66b	6.392b	1,821.720b	181.5^{ns}	13.5^{ns}
	2023	54.0^{ns}	0.38b	3.680b	1,048.800b	174.5^{ns}	12.5^{ns}
Mean	2022	44.95	0.99	9.630	2,746.331	182.15	13.5
	2023	53.57	1.36	13.195	3,760.575	174.00	12.5
Coefficient of variation (%)	2022	0.62	32.20	32.20	32.200	0.22	-
	2023	1.10	124.55	124.55	124.550	0.69	-

[(1)]Means followed by equal letters, in the column, do not differ by Tukey's test, at 5% probability. [(2)]Self-pollinated Arbequina cultivar, used as the control. *Significant at 5% probability. ^{ns}Nonsignificant.

No decrease was identified in the oil percentages of the cultivars with a higher productivity. This finding contrasts with that of Zhang et al. (2022), who observed a reduced oil yield in manually-pollinated oleaginous peony cultivars with a greater productivity, which is understandable since the energy required to produce a fatty acid is quite high and there may be a decrease in oil yield, even if not significant, when the number of fruits is high. In the present work, the quantity of fruits increased due to pollination with Coratina cultivar but without showing a reduction in the percentage of olive oil.

The results obtained in the present study are an indicative that carrying out in-depth studies on olive cultivars is essential to identify which ones present self-compatibility, pseudo-self-compatibility, sporophytic self-incompatibility, and inter-incompatibility, with the aim of modulating and enhancing the production of olive trees in Brazil.

Conclusions

1. The pollination of the Arbequina olive (*Olea europaea*) tree cultivar with pollen from Coratina increases the number of viable fruits and, consequently, favors crop productivity.
2. Cultivars Koroneiki and Pendolino are not suitable pollinators for Arbequina due to inter-incompatibility and a short overlapping period with the flowering of Arbequina, respectively.

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