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Partenocarpia, polinización cruzada y presencia de semillas en mandarina 'Afourer'

Parthenocarpy, Cross Pollination and Seed Presence in 'Afourer' Mandarin

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Abstract

'Afourer' mandarin has been registered as a seedless cultivar. Under Uruguayan productive conditions, in all the evaluated situations, most of the fruits have seeds, which limits their commercial value and marketing. The objectives of this work were to determine 'Afourer' parthenocarpic ability and pollination efficiency of four varieties in commercial plantations. Isolation of cross-pollination with nets in two experiments resulted in 99% of seedless fruits in Paysandú and 100% in San José. In Paysandú, yield components were similar in open-pollination or isolation, demonstrating high parthenocarpic ability. Fruit-set in artificial pollination was 40% or more in all cases. Flowers isolated, emasculated and isolated, and pollinated with 'Afourer' and isolated resulted in seedless fruits. The *in vitro* pollen germination percentage of 'Afourer' mandarin and 'Ortanique' tangor reached 25%, while 'Valencia' orange, 'Lisbon' lemon, and 'Nules Clementine' mandarin, did not exceed 8%. No clear association between these percentages and the number of seeds per fruit was found. The percentage of seeded fruit with artificial pollination was variable, from 18% with 'Lisbon' lemon pollen, to 88% with 'Valencia' orange pollen. These results can be used for new plantation design and agronomical practices implementation to reduce fruit seed number.

Keywords: artificial pollination, emasculation, pollen germination

Resumen

La mandarina 'Afourer', ha sido registrada como una variedad sin semillas. En las condiciones productivas de Uruguay, en todas las situaciones relevadas, la mayor parte de los frutos presenta semillas, lo que limita su precio y comercialización. Los objetivos de este trabajo fueron determinar la capacidad partenocárpica de la mandarina 'Afourer' y determinar la eficiencia polinizadora de cuatro variedades presentes en plantaciones comerciales. En condiciones de aislamiento de polinización mediante mallas, el 99% de los frutos en Paysandú y el 100% en San José no tuvieron semillas. En Paysandú, los componentes del rendimiento fueron similares en condiciones de libre polinización y bajo mallas, demostrando una alta capacidad partenocárpica. Los tratamientos de polinización artificial alcanzaron un porcentaje de cuajado superior a 40% en todos los casos y las flores embolsadas, emasculadas y embolsadas, y polinizadas con 'Afourer' y embolsadas, no presentaron semillas. El porcentaje de germinación de polen *in vitro* de 'Afourer' y 'Ortanique' alcanzó el 25% mientras que el de 'Valencia', limón tipo 'Lisbon' y 'Clementina de Nules', no superó el 8%. No se encontró una asociación clara entre estos porcentajes y el número de semillas por fruto. El porcentaje de frutos con semillas con polinización artificial fue variable, desde un mínimo de 18% con polen de limón tipo 'Lisbon', a un máximo de 88% con polen de naranja.



'Valencia'. Estos resultados sirven de base para el diseño de nuevas plantaciones y medidas de manejo que permitan disminuir el número de semillas.

Palabras clave: emasculación, germinación de polen, polinización artificial

1. Introduction

Uruguayan citriculture is facing the challenge of a varietal replacement, according to the demands of the destination markets of our fruit. The current trend in terms of the development of varieties indicates that one of the main characteristics required in the fruit for fresh consumption is the absence or low number of seeds. The definition of "seedless fruits" has changed over time. In the 80s it was defined commercially as the fruits that had less than five seeds. This concept is considered to be subjective and dependent on consumer expectations and market demand at a given time. Barry (2004), proposes a very strict classification, considering that term exclusively for the occurrence of one seed every 100 fruits. At a commercial level this requirement is generally lower, and for strongly demanded cultivars, for their organoleptic quality, up to one seed per fruit is accepted. Among the varieties introduced in the 90s to the country, 'Afourer' stands out with a few years of commercial production. The variety originated in Morocco, also known as 'W. Murcott' and 'Nadorcott', (Nadori, 2004) and reported as seedless in the absence of cross-pollination, or artificially self-pollinated (Bono and others, 2000; Chao, 2005a). The mandarin 'Afourer' has high fruit quality characteristics and high yields; however, in most plantations, both in the south and north of the country, the presence of seeds is a constant, which limits and decreases their commercial value.

Parthenocarpy, defined as the production of seedless fruits, can be of two types: obligatory or autonomous and stimulative.

The first is defined as the fruit set without any external stimulation, whose best-known examples are the orange 'Washington' navel and the mandarin 'Satsuma'.

The stimulative parthenocarpy requires some promoter factor for the production of fruits. In this case, the stimuli can be pollination, pollen germination, or pollen tube growth without fertilization (Frost and Soost, 1968).

Cross-pollination is one of the central aspects to control when producing seedless fruits, in those self-incompatible cultivars, but that generate viable

gametes. The first studies on self-incompatibility and cross-pollination in hybrid mandarins were already presented in the 60s (Hearn and others, 1969). More recently, under Spain conditions, Soler (1999) and Bono and others (2000), carried out directed manual crossings between the main cultivated species and varieties of the genus *Citrus*, establishing the presence and average number of seeds per fruit according to the source of pollen used.

In parthenocarpic citrus varieties, pollination by other cultivars is mainly entomophilic, with bees being the main pollinating agents.

Chao (2005b), in Californian conditions, determined how far bees can transport pollen from donor varieties, through the use of molecular markers, reaching 500 and 960 meters for pollen of 'Nules Clementine' and 'Afourer' respectively. This poses the difficulties in isolating facultative parthenocarpic cultivars from cross-pollination, under conditions of commercial production. Artificial pollinations in 'Afourer' with pollen from Clementines and hybrids such as 'Fortune', 'Nova' and 'Ortanique' in the conditions of Spain, demonstrate a high capacity for seed formation with all of them (Bono and others, 2000). There are no reports in our country on the parthenocarpic capacity of 'Afourer', nor the pollinating capacity of some of the main commercial varieties present in our plantations.

This study aimed to determine the parthenocarpic capacity of the mandarin 'Afourer' in our agroclimatic conditions and to evaluate the pollinating capacity of different *Citrus* cultivars, in the seed formation of this mandarin.

Material and methods

Parthenocarpy studies. Two experiments were established: (1) In Paysandú department (31° LS), in a frame of 'Afourer' mandarin grafted on *P. trifoliata* L. (Raf.), of two years of age, twenty plants, homogeneous in height and treetop volume, were marked. Half of them, randomly selected, were prevented from cross-pollinating by the use of non-woven fabric mesh, which was fixed on wooden structures placed on the treetops, from the beginning of the budding to the end of the petal fall. The yield was

determined in harvest, and all the fruits of each plant were counted, and in a sample of 50 fruits per plant, the presence and number of seeds per fruit were evaluated. During the set-up period, a temperature and relative humidity sensor (Hygrochron) was installed, with hourly information records in each situation (2). In San José department (35° LS), 10 seven-year-old 'Afourer' plants were selected, taking vigor, treetop volume and health status into consideration, grafted also on *P. trifoliata*, and under fertigation conditions. Two secondary branches were marked in each plant and one of them was protected from cross-pollination, by wire structures lined with tulle mesh, in a period similar to that of experiment 1. In maturation, all the fruits of each branch were collected and the number and percentage of seedless fruits and number of seeds per fruit were evaluated. In both experiments, from the end of the physiological fall (second half of December and first half of January respectively), the equatorial diameter was measured in 10 fruits per plant or five per branch until maturation. *Cross-pollination: setting and presence of seeds.* Experiment 1. In Paysandú (31° LS), in an 'Afourer' frame grafted onto *P. trifoliata* that was seven years old, fifteen plants were selected and, in each one, 60 terminal flower buds were marked, totaling 900 buds.

Six treatments were applied with 150 flowers in each: a) Open pollination (control) b) Bagged flowers c) Emasculated and bagged flowers d) Emasculated flowers, pollinated with 'Afourer' pollen and bagged e) Emasculated flowers, pollinated with 'Nules Clementine' mandarin pollen and bagged f) Emasculated flowers, pollinated with 'Valencia' orange pollen and bagged.

Experiment 2. In the San José's framed group, during the budding and before the anthesis, a total of 450 terminal flower buds were marked in 10 trees, to which three treatments were applied: control, emasculation and artificial pollination with 'Ortanique' tangor pollen and with 'Lisbon' lemon pollen, totaling 150 flowers per treatment. The last two were bagged with tulle meshes for 15 days after pollination. The pollinating varieties were chosen in each area according to the proximity of each to the 'Afourer' frame.

In both experiments, treatments were applied with flowers in state 59 of the BBCH scale (Agustí and others, 1997). The anthers of the donor varieties were collected the day before pollination and kept in the dark at 20°C. Fifteen days after treatments, the bags were removed; every 15 days and until the end of the physiological fall, the setting percentage was

quantified (fruits present/initial flowers x 100); and until maturation, the diameter of the fruits. In harvest, all the fruits were counted, the percentage of final set was determined and the presence and number of seeds per fruit were quantified.

Pollen germination *in vitro*

In every case, an *in vitro* germination test of the different pollen sources used was performed. The flowers were collected from 'Nules Clementine', 'Valencia' and 'Afourer' plants in Paysandú, and from 'Lisbon' lemon and 'Ortanique' in San José. They were placed in an open Petri dish, in a silica gel chamber for 24-36 hours, for the anthers to open. They were kept open in the refrigerator for two hours in order to hydrate the pollen grains. The germination of the pollen grains was carried out on slides, placing a base of 750 µl of solid medium (Brewbaker and Kwack, 1963). Pollen grains were extracted from the anthers, by rubbing them with a fine brush, in order to collect as many as possible. They were placed on the solid medium of each slide.

Subsequently, the pollen grains were covered with 30 µl of the same liquid culture medium. The preparations were placed in a dark chamber at 25°C and 70-80% humidity for 72 hours until the control treatment germinated. Once germinated, the pollen grains were fixed with FAA solution (formaldehyde, acetic, 70% ethanol; 5:5:90). Pollen germination was evaluated by counting an average of 300-400 pollen grains per repetition, with an optical microscope (OLYMPUS ECE-Bi). From the total number of grains counted, the number of germinated pollen grains was determined. The percentage of germination was calculated, taking as the criterion that a pollen grain germinated when the length of the pollen tube exceeded its diameter (Stanley and Linskens, 1974).

The continuous variables were analyzed by the Generalized Linear Model and the proportions by likelihood ratio.

Results and discussion

Parthenocarpic capacity

In isolated pollination conditions, 99% of the fruits in Paysandú and 100% in San José had no seeds (Table 1).

Additionally, the percentage of seedless fruits in the self-pollination treatment (bagging) carried out on individual flowers in Paysandú, reached 100%. These results confirm the reports of Bono and

others (2000) and Chao (2005a) in the conditions of Spain and California respectively, so the presence of seeds in 'Afourer' fruits in our plantations should be explained almost exclusively by the cross-pollination of other varieties. However, under conditions of open pollination, only 34% of the fruits were parthenocarpic in Paysandú, and 29% in San José (Table 1). The harvest results of the Paysandú experiment indicate that the yield components were practically the same in open pollination plants and those isolated from cross-pollination. The maximum and average temperatures recorded under the meshes

were higher than the control plants (Figure 1); these differences, although they advanced flower development by approximately one week, had no impact on the final number of fruits. In the experimental conditions of this research, the parthenocarpic capacity of 'Afourer' was very high, managing to set and develop the same number of fruits as in open pollination conditions. (Table 2). It should be noted that the fruit size was the same for both conditions, without differences depending on the presence of seeds.

Table 1. Percentage of seedless fruits and average number of seeds per fruit in 'Afourer' mandarin plants, under open pollination conditions and mesh-covered, Paysandú and San José. Paysandú, n=50 fruits per plant; San José, all the fruits of each branch.

Situation	Percentage of seedless fruits		Average number of seeds per fruit	
	Paysandú	San José	Paysandú	San José
Open pollination	34 b ^z	29 b	4 a	2,3
Mesh-covered	99 a	100 a	0.04 b	0

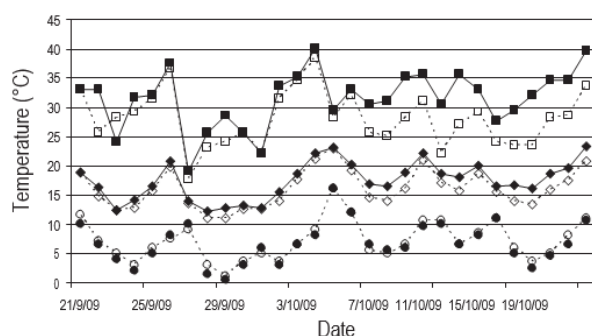
^zDifferent letters in columns indicate significant differences (Tukey, $p \leq 0.05$).

Table 2. Yield components in two-year Afourer mandarin plants, under open pollination conditions and mesh-covered, Paysandú. Data corresponding to 10 plants per situation.

Treatment	Number of fruits/plant	kg/plant	Average fruit weight (g)
Open pollination	117 ns ^z	10.5 ns	88.3 ns
Mesh-covered	115	10.5	87.6

^zNot significant (Tukey, $p \leq 0.05$).

Figure 1. Maximum temperatures (■ mesh-covered and □ control), average (◆ mesh-covered and ◇ control) and minimum (● mesh-covered and ○ control) in 'Afourer' mandarin, experiment of Paysandú.



In contrast, in the San José experiment, the number of fruits per branch under open pollination conditions was significantly higher than that of the mesh-covered branches (27 and 13 respectively). This difference can be explained by the competitive conditions of seedless fruits and seeded fruits on the same plant in San José's trees. 'Afourer' has been reported as a high productivity mandarin in Spain conditions, under open pollination conditions and with seeds presence (Agustí and others, 2005). However, there is no information on their productive capacity in isolation, which can be achieved by separating spatially potential pollinator cultivars, planting cultivars that do not generate viable pollen (e.g. navel oranges) in their vicinity, or covering them with

meshes that prevent bees from accessing during the flowering period (Nadori, 2004).

Pollen germination *in vitro*

The behavior *in vitro* of the pollen of the varieties used and of 'Afourer' was different, being the latter together with 'Ortanique' the ones that reached the highest germination percentage (25%) while 'Nules Clementine', 'Lisbon' lemon and 'Valencia', did not exceed 8% of germination *in vitro*. There is a high variability of this characteristic in different species and varieties of citrus and between years, determining values between 0.6% and 86.3%, (Pardo and others, 2007).

No association was found between the percentage of germination *in vitro* and the presence of seeds in the fruits from these pollinations.

Self-pollination and cross-pollination: setting and presence of seeds.

The percentage of set in all treatments was very high in both experiments, with a minimum in Paysandú of 40% in the pollination treatment with 'Nules

Clementine' and a maximum of 72% in the open pollination control. In both treatments where cross-pollination was prevented (bagged, and emasculated and bagged flowers), the set exceeded 40% and all the fruits obtained were seedless, also indicating, in this case, high parthenocarpic capacity of 'Afourer'. Artificial self-pollination reached the same setting percentages, which allowed postulating that the parthenocarpic of 'Afourer' is autonomous, regardless of the stimulus of pollination (Table 3).

In San José, the lemon pollination treatment (67%) presented the minimum set and, pollinated flowers with 'Ortanique' (78%) presented the maximum, although without achieving significant differences (Table 4).

This high percentage of setting in all treatments can be attributed, in addition to the high parthenocarpic capacity of 'Afourer', to the fact that the shoots used were from terminal flowers, which have a higher setting percentage (daCunha Barros and Gravina, 2006), and that the pollinations were carried out at the end of the flowering period with more favorable temperatures for the setting.

Table 3. Percentage of set, percentage of seedless fruits and average number of seeds per fruit in 'Afourer' mandarin, according to artificial pollination treatments, Paysandú experiment. Number of pollinated and bagged flowers per treatment: 150. The average number of seeds per fruit corresponds to all harvested fruits

Treatment	Setting percentage	Percentage of seedless fruits	Average number of seeds per fruit
Open pollination	72 a ^z	73 c	0.47 b
Bagged	50 b	100 a	0.00 b
Emasculated and Bagged	43 b	100 a	0.00 b
Pollinated with 'Nules Clementine'	40 b	79 bc	0.40 b
Pollinated with 'Afourer'	41 b	95 ab	0.20 b
Pollinated with 'Valencia'	66 a	12 d	2.63 a

^zDifferent letters in columns indicate significant differences (Tukey, $p \leq 0.05$).

Table 4. Setting percentage, seedless fruits percentage, and average number of seeds per fruit in 'Afourer' mandarin, according to artificial pollination treatments, Paysandú experiment. Number of pollinated and bagged flowers per treatment: 140. The average number of seeds per fruit corresponds to all harvested fruits.

Treatments	Setting percentage	Percentage of seedless fruits	Average number of seeds per fruit
Open pollination	70 ns ^z	73 ab	0.34 ns
Pollinated with 'Lisbon' type lemon	67	82 a	0.41
Pollinated with 'Ortanique'	78	68 b	0.41

^zns with no differences; different letters indicate significant differences (Tukey, $p \leq 0.05$).

The 'Valencia' orange pollen was the most efficient pollinator, as it produced the lowest number of seedless fruits and the highest number of seeds per fruit, while 'Nules Clementine' was very inefficient as a pollinator, registering 79% of seedless fruits and a very low number of seeds per fruit (Table 3). The fruits coming from flowers pollinated with 'Lisbon' lemon and 'Ortanique', also presented a high percentage without seeds and a low number of seeds, indicating a low capacity of fertilization of flowers of 'Afourer' (Table 4). There is significant variability in the available results on artificial pollination in 'Afourer', both in the percentage of set and in seed presence. Fang and others (2008) report a 0% set when the flowers were artificially pollinated with 'Valencia Midnight' and 'Delta', which contrasts with the high set verified in our results with the CV 64 clone of 'Valencia'.

In natural pollinations, Agustí and others (2005), also indicate a low efficiency of 'Valencia' orange pollen in promoting the presence of seeds in 'Afourer'. Unexpectedly, in San José, the percentage of seedless fruits and number of seeds per fruit from open-pollinated flowers was similar to that of artificially pollinated flowers, where the amount of pollen applied is much higher and concentrated at the time of greater stigma receptivity (Table 4).

The average number of seeds per fruit, under open pollination conditions in plant isolation experiments in Paysandú and San José (Table 1), was much higher than that recorded in experiments carried out on individual flowers (Tables 3 and 4). This difference can be explained because in the first case the fruits came from all the flowers of the season, while in the second case the fruits came from shoots developed at the end of the season, with much less availability of pollen donor flowers in the nearby varieties.

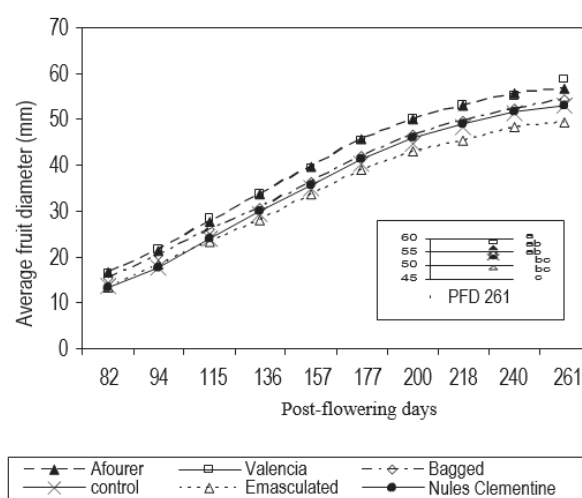
Growth and final fruit size

The evolution of the equatorial diameter of the fruits by pollination treatment in Paysandú is presented in Figure 2. Fruits from open pollination (control) and pollinated with 'Valencia' presented the largest sizes 55 days after the treatments were carried out, maintaining this behavior during most of their development.

During harvest, although the tendency was similar, the statistical differences in the diameter of fruits were confirmed between those coming from flowers pollinated with 'Valencia' and those corresponding to 'Afourer', 'Nules Clementine' and emasculated without pollination, leaving control and bagged ones in an intermediate level. The highest number of

seeds in fruits pollinated with 'Valencia' orange, compared to those with open pollination, did not translate, in this study, into significant differences in their size.

Figure 2. Evolution of the equatorial diameter of 'Afourer' mandarin fruits, by open pollination treatments, bagged, emasculated and bagged, emasculated and artificial pollination with 'Afourer' mandarin, 'Nules Clementine' mandarin, and 'Valencia' orange. Paysandú experiment. Data corresponding to the average of all fruits of each treatment (n=40 to n=72).



Conclusions

Under cross-pollination isolation conditions, 'Afourer' fruits did not present seeds. In the case of individual flowers and at a plant level in Paysandú, the parthenocarpic capacity was very high; in San José, fruit set under mesh was lower than that of open pollination, when both situations competed on the same tree. Of all the donor cultivars used in our work, the orange pollen 'Valencia' was the most efficient, with high percentages of fruit with seed and the highest number of seeds per fruit. 'Nules Clementine', 'Lisbon' lemon and 'Ortanique', were not good pollinators. As there were no differences in the percentage of fruit set between the artificially self-pollinated 'Afourer' flowers and the emasculated and bagged ones, it is postulated that, in this cultivar, parthenocarpy is autonomic. This information is considered relevant when planning the design of the 'Afourer' mandarin plantations, and the implementation of management measures to reduce the number of seeds.

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