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Field spatial pattern of seedy fruit and techniques to improve yield on 'Afourer' mandarin

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ABSTRACT

Cross-pollination increases the number of seedy fruit in a wide range of Citrus cultivars including self-incompatible cultivars such as 'Nadorcott' mandarin, thereby diminishing the commercial value of the crop. Isolated block planting and whole tree net-covering are some of the techniques applied to increase seedlessness of cross-pollinated varieties. However, in net-covered trees a reduction in yield has been reported. The purpose of this work was to study the spatial distribution of seedy fruit at different distances from other pollen sources, and to evaluate the effect of different fruit set promoting techniques applied to covered trees under commercial field conditions. To this aim, four experiments were carried out in a commercial orchard of 'Nadorcott' mandarin to assess: a) the spatial distribution of seed number per fruit and seedy fruit under open-pollinated conditions; b) the effect of girdling and gibberellic acid (GA_3) application on fruit set and seedless fruit in net-covered trees; and c) seedless fruit number in open pollinated trees treated with GA_3 and consecutive copper sulphate ($CuSO_4$) sprays. A gradual reduction in the percentage of seedy fruit from the pollen source up to 140 m away from where the seedless fruit presented a spatially randomized pattern. Net-covered trees reduced the number of seedy fruit and the number of seeds per fruit, whereas the number of fruits was reduced by 66%. Under nets, single GA_3 spray, but not girdling, impaired fruitlet drop. Six consecutive 25 mg per liter or 50 mg per liter sprays of $CuSO_4$ reduced the number of fruit per tree, the 50 mg per liter dose increased fruit seedlessness. Single GA_3 25 mg per liter sprays or combined with $CuSO_4$ reduced the number of seedy fruit with the reduction in fruit per tree.

1. Introduction

Seedlessness in *Citrus* fruit is a desirable trait to improve grower's incomes during commercialization (Vardi et al., 2008). Although many *Citrus* varieties have parthenocarpic ability, it is well known that they can set seedy fruit under cross-pollination conditions. Therefore, in the last decades Citrus breeding programs have been focused on producing seedless varieties either by mutation breeding or supported by biotechnological method (Ollitrault et al., 2007). Besides, different horticultural techniques have also been reported to reduce seedy fruits. Those practices consist of: a) growing Citrus orchard in solid blocks, far away from any Citrus pollen source (Chao et al., 2005); b) avoiding cross-pollination by covering trees with anti-insect nets or by using repellent products to reduced the presence of bees and bumblebees (Rey et al., 2014); c) preventing ovule fertilization through gibberellic acid (GA_3) sprays and thus, accelerating ovule development or impairing pollen tube development through the pistil (Mesejo et al., 2008), and d) avoiding pollen germination or pollen tube growth by copper sulfate ($CuSO_4$) sprays (Mesejo et al., 2006, 2008). These techniques have been tested on trees surrounded by different pollen sources under diverse weather conditions.

'Nadorcott' mandarin produces high yields and good quality fruit, being a well adapted genotype to the agroecological conditions of the Citrus temperate production regions. Under open pollination, 'Nadorcott' produces seedy fruits, reducing the commercial value of the crop; indeed in extreme cases the amount of seeds exceed the requirements for being marketed under the Afourer® trademark (Otero et al., 2014).Therefore, net coverage has become popular in orchards where cross-pollination prevails, avoiding insect pollination irrespective of the distance from any Citrus pollen sources. In this case, an erratic behavior has been achieved regarding to fruit set and size. Gravina et al. (2011), covering 'Nadorcott' branches with nets, reported a reduction in fruit set in the northern and warmer zones of Uruguay but not in the southern and cooler region, whereas whole-tree net coverage showed to reduce fruit set and yield (Otero and Rivas, 2010). This behavior could be explained by higher air temperature and a reduction of photosynthetic rate, triggering the fruitlet abscission process (Cary and Weerts, 1978) under the nets. Additionally, fruit size is an important

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trait that affects crop value. Seedless fruit are usually smaller in size and weight; indeed fruit size of 'Nadorcott' has also been directly related with the number of seeds (Chao, 2005). However, Gravina et al. (2011) found no relationship between fruit size and seed number in the northern area of Uruguay.

It is widely known that GA_3 sprays during petal fall can improve fruit set in several self-incompatible parthenocarpic Citrus cultivars such as Clementines (Talon et al., 1992), 'Minneola', 'Nova', 'Orlando', 'Osceola' and 'Robinson' hybrids (Krezdorn and Jernberg, 1977; Goren et al., 1992). In seeded Citrus cultivars, pollination stimulates GA_1 synthesis in developing ovaries and thus an increases in fruit set is achieved (Ben-Cheikh et al., 1997). In addition, fruit set in Citrus can also be promoted by trunk or branch scoring or girdling from full-bloom until 35 d after anthesis (Goren et al., 2003; Rivas et al., 2006, 2007). Girdling has been reported to raise canopy carbohydrate availability, supporting fruitlet growth and final fruit set (Rivas et al., 2006, 2007).

The purpose of this work was to study the field spatial pattern of fruit with seeds in open-pollinated 'Nadorcott' mandarin trees, and to evaluate the effect of different horticultural techniques to reduce seedy fruit of 'Nadorcott' mandarin on yield and fruit quality under commercial field conditions.

2. Materials and methods

Experiments were conducted in a 4.4 ha orchard of 7-year-old 'Nadorcott' mandarin trees [*Citrus reticulata* Blanco] budded onto *Poncirus trifoliata* (L.) Raf. spaced at 4 × 7 m in Salto, Uruguay (30°57′06.6"S, 57°50′16.8"W). Trees were grown in a sandy-loamy soil of 80 cm depth, with a bulk density of 1.71 g cm^{-3}, field capacity (−0.01 MPa) of 0.29 m^3 m^{-3} and a wilting point (−1.5 MPa) of 0.15 m^3 m^{-3} within the 40 cm topsoil. The selected orchard was surrounded at the south by 'Murcott' mandarin, at the east and north by 'Valencia' low seedlessness cultivar, and from the west by native flora (Fig. S1).

2.1. Experiment 1. Field spatial pattern of seedy fruit in open pollinated 'Nadorcott' mandarin trees

The 4.4 ha 'Nadorcott' mandarin orchard was divided in to a grid containing 10 trees in each cell. One week before harvest, one tree per cell was selected; georeferenced by GPS (Leica, USA) and then 100 fruit from each tree were randomly harvested to record the number of seed per fruit. The percentage of seedy fruit and the mean number of seeds per fruit per tree was spatially analyzed. A total of 138 trees were sampled.

Global spatial dependence analysis was performed using Morańs index (Moran, 1948). Morańs index (I) was calculated as follows:

$$I = \frac{N \sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij} (X_i - \overline{X}) (X_j - \overline{X})}{(\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij}) \sum_{i=1}^{n} (X_i - \overline{X})^2}$$

References : N: number of observations; $\overline{X}$: mean of the variable; Xi: the variable value at a particular location; Xj: the variable value at another location; Wij: is a weighted indexing location of i relative to j

Moran's index computes the degree of spatial autocorrelation between the values of a variable as a function of spatial lags over some range of distances (Moran, 1948). When spatial dependency is present, the measurements will tend to be more similar to measurements taken nearby than to measurements taken farther away.

GS+ v 9.0 software (Gamma Design Software, USA) was used to model the semivariograms to estimate the parameters of the variance-distance models and to interpolate the data using the stochastic interpolation (prediction) method of the universal Kriging. Universal Kriging was chosen to avoid possible and expected trends in the data, due to the spatial direction to the pollen sources. The criteria to choose the best fitted variogram model was the highest r^2 obtained between observed and predicted values. The lag size used for both variables was 15 m. ArcGIS 9.0 (Esri, USA) software was used for graphical representation.

2.2. Experiment 2. The effect of anti-insect net on seed content in 'Nadorcott' mandarin

During the following spring (September), three treatments were carried out in the same selected plot of Experiment 1, using six trees per treatment with similar health, vigor and previous yield. In the first treatment, trees were completely covered with a 50 mesh-net cloth in order to avoid cross pollination. Nets were placed at the beginning of sprouting (stage 01 of the BBCH *Citrus* phenological scale, Agustí et al., 1997) and removed 10 days after petal fall. In the second treatment, in order to increase air temperature (Ta), trees were covered in the same way and time as the first treatment, but additionally 1 m wide 100 µm polyethylene nylon was placed over the net. Non-covered trees were used as control trees.

Air temperature and humidity sensors were placed between trees in each treatment and data were recorded every 10 min using Hobo dataloggers (HOBO, Pro Serie, USA).

Before sprouting, 20 shoots per tree were selected in five trees per treatment. Shoots were 30–35 cm long and the number of nodes for each shoot was recorded. Twice a week, the phenological stage (BBCH phenological scale for Citrus) was assessed by recording the number of flowers in each stage. Time course of flowering was expressed as the average number of flowers at each phenological stage per 100 nodes.

At harvest, all fruit from each tree were harvested and seed number per fruit was recorded. Results were expressed as the number of seeds per fruit per tree and the percentage of fruit with seeds per tree.

Statistical design and data analysis. A completely randomized design with six replicates was used in the experiment; each tree represented one replicate. SAS software (SAS Inst. Inc., Cary, N.C.) was used for data analysis. Means were separated by Duncan's Multiple Range Test (DMRT) at $p < 0.05$.

2.3. Experiment 3. Techniques to reduce the seed number in 'Nadorcott' mandarin

In the same orchard of Experiments 1 and 2 and concomitantly with Experiment 2, 54 open-pollinated trees were chosen having similar health, vigor and previous yield. Six treatments were carried out in open-pollination trees:

a) Consecutive foliar spray of commercial $CuSO_4$ ($CuSO_4.7H_20$) at 25 mg L^{-1} was applied. Trees were sprayed six times at 3 d intervals starting when 20% of the flowers were at anthesis (stage 61 of the BBCH phenological scale for Citrus).
b) Copper sulfate spray at 50 mg L^{-1} in the same condition and timing as in treatment 'a'.
c) Single foliar spray of GA_3 at 25 mg L^{-1} when 50% of the flowers reached stage 59 of the BBCH phenological scale, corresponding to closed and elongated flowers.
d) Single foliar spray of GA_3 at 50 mg L^{-1}, at the same timing as in treatment 'c'.
e) Single foliar spray of GA_3 at 50 mg L^{-1} (stage 59 of the BBCH phenological scale) and subsequently six periodical $CuSO_4$ sprays at 25 mg L^{-1} as in treatment 'a'.
f) Control trees without any chemical spray.

Each single GA_3 spray solution was adjusted to pH 5.5 and it was applied at 4 L per tree, totalizing 36 L spray solution in 9 trees. For the consecutive $CuSO_4$ treatment, the solution was also sprayed at 4 L per tree in each single application, which totalizes 216 L of spray solution for the 9 trees and the 6 consecutive applications. During the ripening