Europ.J.Hort.Sci., **69** (4). S. 244–249, 2004, ISSN 1611-4426.

# Influence of *Citrus aurantium* L. and *Poncirus trifoliata* (L.) Raf. Rootstocks and Nutrient Sprays on Granulation of Valencia Sweet Orange [*C. sinensis* (L.) Osbeck] Fruits

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## Summary

Experiments on the influence of rootstocks and foliar sprays of macro-and microelements on granulation and quality of Valencia sweet orange (*Citrus sinensis* (L.) Osbeck) fruits were carried out over a three-year-period (1996–1998) at the Agios Saranda Pomology Research Station. The *Poncirus trifoliata* rootstock markedly reduced granulation with 31 % compared with 73 % on *C. aurantium* rootstock.

The combined spraying with zinc, + manganese and copper (each 0, 5 %) was the best treatment in reducing granulation and enhancing fruit quality. This combination reduced granulation on both rootstocks to about 12–41 % compared to that of control treatment. Zinc or copper separately reduced the incidence of granulation to almost 24 %–28 % compared to the unsprayed control treatment on both rootstocks. Potassium nitrate (1 %), calcium hydroxide (2 %) and boric acid (100 mg $l^{-1}$) reduced granulation to a lesser extent.

Most of these treatments improved fruit quality and resulted in much more endocarp juice, total soluble solids, and vitamin C content than the control and also markedly reduced peel weight and acidity. The use of specific rootstocks and foliar application of nutrients is a powerful tool in reducing granulation and in improving fruit quality in sweet Valencia orange.

## Zusammenfassung

**Einfluss von Unterlagen [*Citrus aurantium* L. und *Poncirus trifoliata* (L.) Raf.] und Blattdüngung auf die Granulierung von Valencia Apfelsinen [*C. sinensis* (L.) Osbeck].** Während einer dreijährigen Versuchszeit (1996–1998) wurde der Einfluss von Unterlagen und Blattspritzungen mit verschiedenen Makro- und Mikronährstoffen auf die Granulierung und Qualität von Valencia Apfelsinen (*C. sinensis* (L.) Osbeck ) im Agios Saranda Obstbauforschungsinstitut untersucht. Die Veredelung auf P. *trifoliata* verminderte die Granulierung der Früchte um 31 %, während die Verwendung vom *C. aurantium* als Unterlage zu einer Verringerung von nur 73 % führte.

Die kombinierte Spritzung mit 0,5 % Zinksulfat, 0,5 % Mangansulfat und 0,5 % Kupfersulfat bewährte sich als die beste Behandlung für die Reduzierung der Fruchtgranulierung und die Verbesserung der Fruchtqualität. Diese Behandlung verminderte die Fruchtgranulierung auf ca. 29–31 % im Vergleich zu der Kontrollvariante. Die Anwendung von nur 0,5 % Zinksulfat oder 0,5 % Kupfersulfat Spritzlösung reduzierte das Auftreten der Fruchtgranulierung um ca. 24–28 % in beiden Unterlagen im Vergleich zu der unbehandelten Kontrollvariante. Die Spritzung mit 1 % Kaliumnitrat, 2 % Calciumhydroxid und 100 mg $l^{-1}$ Borsäure reduzierte ebenfalls das Auftreten von Fruchtgranulierung zu einem geringeren Prozentsatz.

Die meisten von diesen Veredelungs- und Blattspritzungsbehandlungen verbesserten die Fruchtqualitat, erhöhten die Saftausbeute und die Konzentration an wasserlöslicher Trockensubstanz und Vitamin C im Fruchtendocarp im Vergleich zu der Kontrollvariante. Darüber hinaus senkten sowohl die Veredlung auf die zwei getesteten Unterlagen als auch die Blattspritzungen deutlich das Schalengewicht der Apfelsinenfrucht. Diese Ergebnisse deuten darauf hin, dass sowohl die Veredlung auf bestimmte Unterlagen als auch die Blattdüngung, effektive Maßnahmen für die Verminderung der Fruchtgranulierung und die Erhöhung der Fruchtqualität bei Valencia Apfelsinen sind.

**Key words.** granulation – sweet orange – nutrient sprays – rootstocks – fruit quality.

## Introduction

Valencia sweet orange (*C. sinensis* (L.) Osbeck) is characterised by late ripening, good flavour, high sugar, and yield and quality juice without limorine. These characteristics make Valencia oranges excellent for juice production and also for fresh fruit consumption. It is one of the sweetest orange species in many citrus growing areas. Its worldwide distribution marks it as one of the most commercially interesting sweet orange species.

Despite the many good characteristics of Valencia orange, there are some disadvantages. One of which is fruit granulation later in the season, a serious problem in most citrus-growing countries. During granulation, the juice sacs become hard and dry, the fruit becomes unmarketable, and the growers suffer heavily. Granulation unlike splitting and creasing is not noticeable externally. Fruits develop the typical hardened juice vesicles, but may have collapsed juice vesicles as well. Increase in cell wall thickness (BURNS and ACHOR 1989) and respiration rate (BURNS 1990) are associated with the hardening of the juice vesicles. Many efforts have been made in order to solve this problem but without much success.

Limited success has been reported by BARTHOLOMEW et al. (1941) and AWASTHI and NAURIYAL (1972) with calcium application, and by SINGH and SINGH (1981) with foliar spray of various macro- and microelements. In the development of this phenomenon, environmental conditions and the rootstock also have some influence. Granulation is strongly affected by rootstock vigour, the climate, the soil humidity, and relative humidity. It has been observed that granulation of different cultivars varies when these are grafted on different rootstocks. Rootstocks characterised by low vigour have a less positive effect on granulation (Protopapadakis, personal communication).

The incidence of granulation was found to be higher on trees, which were deficient in various macro and microelements (AWASTHI and NAURIYAL 1972). The translocation of photosynthetic products towards the fruits is restricted during granulation, (DROSOPOULOS, 1998). Potassium and microelements such as boron, zinc, and copper have been reported to facilitate this translocation (EATON 1952; SINGH and SINGH 1981). Therefore, these elements could be a means of reducing granulation. The purpose of this study was to investigate the possible control of granulation by the use of two rootstocks and of some macro-and micronutrients applications in different concentrations and combinations.

## Materials and Methods

The research was conducted in an orchard at the Agios Saranda Pomology Research Station. One hundred and ninety two Valencia orange trees (cv. 'Frost'), 22-year-old were used, which were grafted on two rootstocks *Citrus aurantium* L. and *Poncirus trifoliata* (L.) Raf. at 5x5 m distance. The type of soil was sandy clay with 35–38 % clay and 50–53 % sand, pH=7.7–7.8 and $CaCO_3$ 10.3. The soil was rich in phosphorus (P) with medium levels in potassium (K) and nitrogen (N). Nutrient analysis in leaves before treatments application showed that the levels of microelements were low (Cu 4–5 ppm, B 20–23 ppm, Mn 18–20 ppm and Zn 16–17 ppm) although no visible deficiency symptoms were observed of any of the above nutrients (Cu, B, Mn or Zn).

For each rootstock during the years 1996–1998 the experiment was carried out using a completely randomised block design with three blocks per treatment.

Each block consisted of 4 well-developed trees of similar height with 12 trees for each treatment and rootstock. Eight treatments were applied for each rootstock: Control (water), Calcium hydroxide 2 %, Potassium nitrate 1 %, Zinc sulphate 0.5 %, Manganese sulphate 0.5 %,

Copper sulphate 0.5 %, Zinc sulphate 0.5 % + Manganese sulphate 0.5 % + Copper sulphate 0.5 %, Boric acid 100 mg $l^{-1}$. Nutrients were sprayed 3 times at monthly intervals starting from October. The treatment solution was sprayed to run off on each experimental tree (10 l/tree spray solution approximately).

1. The incidence of granulation was expressed as the percentage of fruits displaying the disorder per total fruits collected at harvest in (15–20) August of each year by cutting 12 fruit from each experimental tree and from each side (North, South, East and West).
2. Ten fruits from each experimental tree were randomly collected and fruit fresh weight, peel and endocarp weight and juice volume were measured.
3. The total volumetric acidity of the juice was determined by titration with 0.1 N NaOH and expressed as % citric acid. The chemical method for this determination is based on the conversion of coloured 2,6 dichlorophenol indophenol to the colourless form.
   a. Total soluble solids mostly in the form of sugars were determined using a hand refractometer and expressed in °Brix.
   b. Fruit fresh weight was measured and fruit was then placed in an oven at 70 °C until constant weight. Dry weight was expressed as % of fresh weight.

The data was analysed by analysis of variance (one-way ANOVA) with mean separation by Duncan's multiple range test at p=0,05. Statistical analysis was performed using Statgraphic Plus Version 2.1 for Windows.

## Results

*Effect of rootstocks and nutrient sprays on granulation*

Fruits of the trees grafted on *P. trifoliata* rootstock exhibited less granulation than fruits of trees grafted on *C. aurantium* (Table 1). This effect was observed during the three years of the experiment. All nutrient sprays of macro-and microelements on both rootstocks reduced the granulation of fruits markedly. In trees grafted on rootstocks *C. aurantium*, the minimum incidence of granulation resulted from the combination spray of zinc sulphate + manganese sulphate + copper sulphate with 41 % of fruits exhibiting granulation in comparison to 73 % of fruits from control trees. Similar results were given by of zinc sulphate or copper sulphate, which reduced granulation to 28 % and 25 % in comparison to the control, respectively.

Table 1. Influence of rootstocks *Citrus aurantium* and *Poncirus trifoliata* and nutrient spray on incidence of granulation of Valencia sweet orange (mean value of three years).

| Treatment and concentration | *C. aurantium* Incidence of granulation ( %) | *P. trifoliata* Incidence of granulation ( %) |
|---|---|---|
| Control (water) | 73 a | 41 a |
| Calcium hydroxide 2 % | 62 b | 21 cb |
| Potassium nitrate 1 % | 55 bc | 28 b |
| Zinc sulphate 0.5 % | 45 c | 24 c |
| Manganese sulphate 0.5 % | 58 bc | 26 b |
| Copper sulphate 0.5 % | 49 c | 17 bc |
| Zinc sulphate 0.5 % + manganese sulphate 0.5 % + copper sulphate 0.5 % | 41 cd | 12 c |
| Boric acid 100 mg $l^{-1}$ | 59 bc | 27 b |

1. Duncan test was used for the comparison of means. Level of significant difference P=0.05.
2. The three applications dates were 1st October, 1st November, and 1st December of each year.

Table 2. Influence of nutrient spray on fruit weight (endocarp, peel and endocarp : peel ratio) of the Valencia orange on two rootstocks (mean value of three years).

| Rootstock | Treatment and concentration | Fruit weight (g) | Endocarp weight (g) | Peel weight (g) | Endocarp : peel ratio |
|---|---|---|---|---|---|
| *Citrus aurantium* | Control (water) | 159.5 a | 117.9 a | 41.6 a | 2.81 : 1 a |
| | Calcium hydroxide 2 % | 160.1 a | 119.6 ab | 40.5 ab | 2.95 : 1 ab |
| | Potassium nitrate 1 % | 159.1 a | 122.8 bc | 36.3 c | 3.38 : 1 c |
| | Zinc sulphate 0.5 % | 160.5 a | 119.9 ab | 40.6 ab | 2.81 : 1 a |
| | Manganese sulphate 0.5 % | 160.2 a | 121.2 b | 39.0 b | 3.1 : 1 b |
| | Copper sulphate 0.5 % | 160.0 a | 121.4 b | 38.6 b | 3.14 : 1 b |
| | Zinc sulphate 0.5 % + manganese sulphate 0.5 % + copper sulphate 0.5 % | 159.7 a | 121.9 b | 37.8 b | 3.22 : 1 b |
| | Boric acid 100 mg $l^{-1}$ | 160.6 a | 118.1 a | 42.5 a | 2.77 : 1 a |
| *Poncirus trifoliata* | Control (water) | 168.1 a | 122.3 a | 45.8 a | 2.67 : 1 a |
| | Calcium hydroxide 2 % | 168.3 a | 124.0 ab | 44.3 ab | 2.79 : 1 ab |
| | Potassium nitrate 1 % | 168.2 a | 125.9 b | 42.3 b | 2.97 : 1 b |
| | Zinc sulphate 0.5 % | 168.3 a | 122.6 a | 45.7 a | 2.68 : 1 a |
| | Manganese sulphate 0.5 % | 168.5 a | 125.4 b | 43.1 ab | 2.9 : 1 b |
| | Copper sulphate 0.5 % | 168.7 a | 125.7 b | 43.0 b | 2.92 : 1 ab |
| | Zinc sulphate 0.5 % + manganese sulphate 0.5 % + copper sulphate 0.5 % | 168.0 a | 127.1 bc | 40.1 c | 3.1 : 1 c |
| | Boric acid 100 mg $l^{-1}$ | 168.0 a | 123.8 ab | 44.2ab | 2.8 : 1 ab |

1. Duncan test was used for the comparison of means. Level of significant difference P=0.05.
2. The three applications dates were 1st October, 1st November, and 1st December of each year.

Potassium nitrate and boric acid reduced the incidence of granulation (14–18 % reduction), and similar results were observed with the application of calcium hydroxide 2 %. In trees of Valencia orange grafted on the rootstock *P. trifoliata*, the combined spraying of zinc sulphate + manganese sulphate + copper sulphate alone was the best treatment, resulting in a marked reduction of granulation at about 29 % over the control. Copper sulphate and calcium hydroxide also reduced the granulation level to 17 % and 21 % respectively compared with 41 % of granulated fruits in trees of the control treatment and were followed by manganese sulphate, boric acid and potassium nitrate.

*Effect of rootstocks and nutrient sprays on fruit characteristics*

Most of the treatments in both rootstocks markedly reduced the peel and increased the pulp of fruit (Table 2). Concerning the rootstock *C. aurantium*, a spray with potassium nitrate 1 % resulted in lower peel weight and

higher weight (g) of pulp per fruit than those of the control treatment. Spray with manganese sulphate or copper sulphate alone and the combination of zinc sulphate, manganese sulphate or copper sulphate gave almost similar results.

On the rootstock *P. trifoliata*, a spray with the combined solution of zinc sulphate 0.5 %, manganese sulphate and copper sulphate gave statistically lower fruit peel weight, almost 40.1 g than 45.8 g of the control and higher fruit pulp weight almost 127.1 g than the control with 122.3 g. The application of potassium nitrate 1 % gave almost similar results. Considering fruit weight, there was no statistically significant difference between treatments on either rootstock.

*Effect of rootstocks and nutrient sprays on qualitative characteristics of fruit*

Almost all treatments resulted in a marked increase of juice content per fruit (ml) on both rootstocks (Table 3). On the rootstock *C. aurantium*, the spraying of combined zinc sulphate, manganese sulphate and copper sulphate resulted in high juice content per fruit with 65.3 ml while the control treatment resulted in only 59.25 ml juice per fruit. High juice content per fruit was also obtained by spraying with copper sulphate and zinc sulphate separately (Table 3). Spraying trees grafted on the rootstock *P. trifoliata,* combining three microelements, gave higher juice content per fruit (almost 66.7) than the control. Similar results were obtained by spraying with copper sulphate and zinc sulphate separately. There was no statistically significant difference between the other treatments regarding the juice content per fruit.

Acidity on both rootstocks was reduced by spraying with the combination of manganese sulphate + zinc sulphate, sulphate + copper sulphate to almost 1.06 % in fruits of trees grafted on *C. aurantum* and to 1.05 % in fruits of trees grafted on *P. trifoliate*, while the control trees showed 1.30 % and 1.35 %, respectively (Table 3).

In rootstock *C. aurantium*, potassium nitrate and boric acid produced similar reductions in acidity, as did boric acid and calcium hydroxide in *P. trifoliata*.

Total soluble solids of fruits increased on both rootstocks with the combined spraying of three microelements to 0.37 % and 0.33 %, respectively. Separate spraying of zinc sulphate and boric acid 100 mg $l^{-1}$ gave almost similar results (Table 3).

Vitamin C increased markedly during the three-year experiment period on the two rootstocks. Spraying with the combination of three microelements gave the highest vitamin C content per fruit on both rootstocks (4.90 mg 100ml$^{-1}$ and 6.10 mg 100ml$^{-1}$ of vitamin C respectively) and was followed by potassium nitrate, which gave almost similar results (Table 3). Concerning the fruit dry weight, there was not any significant difference between treatments and rootstocks.

## Discussion

Valencia orange grafted on *P. trifoliate* showed less granulation (almost 32 %) than grafts on *C. aurantium*. *C. au-*

Table 3. Influence of nutrient spray on fruit juice qualitative characteristics of Valencia orange on two rootstocks (mean value of three years).

| Rootstock | Treatment and concentration | Juice content per fruit (ml) | Acidity (%) | Total soluble solid content (Brix) | Vitamin C (mg 100 ml$^{-1}$) | Dry weight |
|---|---|---|---|---|---|---|
| *Citrus aurantium* | Control (water) | 59.3 a | 1.30 a | 8.2 a | 61.95 a | 10.03 a |
| | Calcium hydroxide 2 % | 60.1 ab | 1.19 ab | 8.41ab | 65.15 ab | 10.07 a |
| | Potassium nitrate 1 % | 60.2 ab | 1.15 ab | 8.31 a | 67.35 b | 10.03 a |
| | Zinc sulphate 0.5 % | 63.1 b | 1.07 c | 8.46 ab | 63.2 a | 10.05 a |
| | Manganese sulphate 0.5 % | 60.0 ab | 1.23 ab | 8.50 b | 62.55 a | 10.08 a |
| | Copper sulphate 0.5 % | 64.7 bc | 1.10 bc | 8.43 ab | 64.3 ab | 10.11 a |
| | Zinc sulphate 0.5 % + manganese sulphate 0.5 % + copper sulphate 0.5 % | 65.3 c | 1.06 c | 8.57 b | 66.85 b | 10.10 a |
| | Boric acid 100 mg $l^{-1}$ | 60.1 ab | 1.14 b | 8.58 b | 63.95 a | 10.07 a |
| *Poncirus trifoliata* | Control (water) | 60.2 a | 1.35 a | 8.31 a | 60.91 a | 10.05 a |
| | Calcium hydroxide 2 % | 63.7 b | 1.21 ab | 8.54 ab | 64.20 ab | 10.06 a |
| | Potassium nitrate 1 % | 63.9 b | 1.26 a | 8.57 ab | 66.72 b | 10.11 a |
| | Zinc sulphate 0.5 % | 64.7 bc | 1.11 c | 8.63 b | 63.41 ab | 10.10 a |
| | Manganese sulphate 0.5 % | 61.1 ab | 1.19 abc | 8.39 a | 62.49 a | 10.08 a |
| | Copper sulphate 0.5 % | 66.5 c | 1.09 c | 8.56 ab | 63.53 ab | 10.08 a |
| | Zinc sulphate 0.5 % + manganese sulphate 0.5 % + copper sulphate 0.5 % | 66.7 c | 1.05 d | 8.64 b | 67.0 b | 10.09 a |
| | Boric acid 100 mg $l^{-1}$ | 61.2 ab | 1.18b | 8.66 b | 63.59 ab | 10.06 a |

1. Duncan test was used for the comparison of means. Level of significant difference P=0.05.
2. The three applications dates were 1st October, 1st November, and 1st December of each year.

*rantium* is a vigorous rootstock, which gives trees a larger canopy than those grafted on *P. trifoliata*, which are characterised as dwarfing. Dwarf trees are characterised by larger quantities of organic and inorganic nutrients than vigorous ones (COLBY 1930; RAO and BERRY 1940; WATCHER and SCHULL 1976). Total soluble solids (TSS) in granulated fruit (data not shown) was 1–1.5 % lower than control (8,2 %) on a vigorous rootstock as *C. aurantium,* while on a low vigour rootstock as *P. trifoliata* TSS was 0,5–0,8 % lower than control (8,31 %). These differences in TSS are associated with the percentage of granulation that was higher in *C. aurantium* than *P. trifoliata*. ERICKSON (1968) reported that fruit with lower normal TSS as in cultivars on vigorous rootstocks like rough lemon are more prone to granulation. The fact that TSS were found less than 10 % is probably due to the relatively low temperatures in this area, which affect negatively photosynthesis rate on the trees and therefore sugar accumulation in fruits. The high P level found in the soil might also decrease TSS as has been reported previously (EMBLETON et al. 1971).

Nutrient macro-and microelements play a vital role in the various physiological functions of the plant and are necessary for the development of fruits. There are observations that citrus-plants, which are deficient in certain elements, present increased incidence of granulation (SINGH and SINGH 1981). Ripening of fruits takes place late in spring until summer simultaneously with the flowering shoot development, which presents a strong sink of nutrient elements and organic compounds. In conclusion, an insufficient level of mineral nutrients is frequently observed, which has negative influence on the development of granulation in the fruits.

The combined spray of three microelements zinc sulphate + manganese sulphate + copper sulphate proved to be the best treatment in reducing the granulation. Zinc deficient plants of citrus have shown reduction in auxin content and an increase in peroxidase activity.

The zinc application in our case may have overcome the effects of auxin deficiency. Manganese deficiency reduced the level of lignin in trees, and caused a reduction in photosynthetic activity and disorder in the absorption of $O_2$ (DROSOPOULOS 1998).

BULD et al. (1953), concluded that the highest level at which copper deficiency is expressed during July–August, is <5 ppm (on d.w. basis) on leaves. Copper has been reported to increase the synthesis of simple sugars. The combined spray of microelements (Zn, Mn, and Cu) decreased starch content and increased soluble sugars, which resulted in a reduction of granulation.

Potassium nitrate proved to have a positive effect in reducing granulation. NIGHTINGALE et al. (1930) reported that a low potassium level in leaves of the citrus resulted in accumulation of starch and lignin while HART (1934) and EATON (1952) found increased respiration and low assimilation rate and decreased sucrose content. Therefore, the effectiveness of potassium spray on reducing granulation and giving an improvement in the fruit quality is justified.

The application of calcium hydroxide markedly reduced granulation and improved fruit quality. PALEG (1960) has reported that Ca increases amylase activity and increases sugar content, which have been associated with reduction of granulation and when the leaves have adequate calcium content resulting in limited fruit malformations (DROSOPOULOS 1998). The beneficial effect of calcium application in reducing granulation has been reported by BARTHOLOMEW et al. (1941) and AWASTHI and NAURIYAL (1972).

Boron has been reported to regulate the translocation of sugars (DUGGER and HUMPHRIES 1957) and to facilitate the movement of sugar in fruits by forming more permeable boron-sugar complexes (GAUCH and DUGGER 1953; SISLER et al. 1956). SINGH and SINGH (1981) reported that healthy citrus fruits have a considerably higher amount of B than granulated ones. CALLAN et al. (1978) has reported that B is translocated to fruits by the near branches and not through roots. Therefore, the possibility of B application in reducing the incidence of granulation and thick rind of granulated fruits could possibly be associated with boron deficiency in the experimental trees. The effect of foliar nutrient spray application on fruit quality in citrus even in the absence of visible deficiency symptoms has been reported by PARDEEP and SHARMA (1997).

*Conclusions*

From the data presented, it can be concluded that rootstocks could be a possible means of controlling granulation. Dwarf rootstocks cause lower granulation percentage than vigorous ones.

A foliar spray of nutrients reduced the incidence of granulation and improved fruit quality. In order to overcome this problem in citrus fruits, nutrient sprays must be applied many months before granulation actually appears, because it is very likely that the factors responsible for the disturbance in the nutrient balance are activated many months before granulation appearance.

However, there is a pressing need for further studies on the effect of other rootstocks, of other nutrients in different concentrations and combinations as well as experiments on the timing of application.

## Acknowledgements

I thank the Institute of Pomology for the financial support during the experiment.

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Received August 21, 2003 / Accepted May 17, 2004

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