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Effects of girdling, nitrogen, zinc and auxin foliar spray applications on mandarin fruit "Nova" quality characteristics

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Abstract: Four treatments were applied in young mandarin trees, variety "Nova", in order to assess their effects on fruit quality. The treatments comprised an untreated control, applications of zinc plus nitrogen, post-bloom auxin application and pre-bloom girdling. At harvest the physical quality characteristics of the fruits were measured (weight, diameter, length, peel thickness, juice content, peel color) along with juice quality characteristics (pH, total soluble solids, titratable acidity, phenolic compound content, antioxidant capacity and carbohydrates). Zinc and nitrogen as well as auxin application resulted in the heaviest fruits among treatments, while juice content was the lowest under control treatment. None of the treatments had a significant effect on maturity index of the fruit. Girdling slightly increased the antioxidant capacity of the juice while decreased peel color index. Sucrose, glucose and fructose concentration was highest in fruits from girdled scaffolds, resulting in the highest sweetness index, while the lowest was observed under zinc and nitrogen application. It seems that all treatments enhanced fruit quality, but girdling had a distinctive and significant effect on most of the fruit quality characteristics assessed.

Key words: Antioxidant capacity, carbohydrates, fruit quality, phenolic compounds, triclopyr

تأثير التحليق وإضافة السماد النيتروجيني والزنك والرش الورقي للأوكسين على خصائص جودة أشجار اليوسفي "نوفا"

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الملخص:تم تطبيق أربع معاملات عل أشجار اليوسفي صنف "نوفا "، من أجل تقييم تأثرها على جودة الثمار .المعاملات تضمنت معاملة الشاهد، المعاملات كانت إضافة الزنك بالإضافة إلى النيتروجين، إضافة الأوكسين بعد التزهير والتحليق قبل التزهير.عند الحصاد تم قياس الخصائص الفيزيائية للجودة الفواكه (كالوزن ، والقطر ، والطول ، سمك القشرة ، ومحتوى العصير ولون القشرة) جنبا إلى جنب مع خصائص نوعية عصير (الرقم الهيدروجيني والمواد الصلبة الذائبة الكلية، الحموضة، محتوى المركبات الفينولية، والقدرة المضادة للأكسدة والكربوهيدرات).اظهرت النتائج ان معاملات الزنك والنيتروجين وكذلك إضافة الأوكسين اعطت أعلى وزن للثمرة من بين المعاملات، في حين أظهرت معاملة الشاهد اقل محتوى العصير.كما لم تظهر النتائج اي فروق معنوية على مؤشر نضج الثمرة .التحليق ساهم في زيادة طفيفة في القدرة المضادة للأكسدة للعصير بينما انخفض مؤشر لون القشرة . وكان تركيز السكروز والجلوكوز والفركتوز في الفاكهة أعلى في معاملة التحليق، مما أدى إلى أعلى مؤشر حلاوة، في حين لوحظ أدنى مؤشر تحت معاملة الزنك والنيتروجين.كل المعاملات أظهرت تحسن في جودة الثمار، ولكن التحليق كان لها تأثير معنوي كبير على خصائص جودة الثمار.

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Introduction

Citrus is one of the most important fruit tree species in the world, as the fruits are a valuable source of nutrients, vitamins and other antioxidant compounds. It is presumed that during the growing season flowers and fruits compete one another and with vegetative growth for plant metabolites (Rivas et al., 2007). This competition hypothesis is based mainly on the carbohydrate supply and reserves, which regulate fruit set and subsequent fruit drop (Rivas et al., 2006). Fruit set and growth requires large amounts of carbohydrates, which are provided by the photosynthesis of the current season's leaves and/or by the reserves accumulated during the winter. If the requirements exceed the capacity of the tree to supply assimilates, fruitlet abscission is triggered, in order to adjust the final fruit load to carbohydrate supply.

Among agricultural practices which may improve carbohydrate balance and increase their availability is girdling, which increases fruit set and yield (Peng and Rabe, 1996). It has been found that cytokinin and gibberellin content of the shoots are modified along with the C/N ratio, which increases (Li et al., 2003). Spring girdling, i.e. pre-bloom removal of a wide strip of bark without injuring the xylem, is widely used in citrus species mainly to increase fruit set and size as well as fruit quality (Mostafa and Saleh, 2006).

Among the minerals which play significant role in initial fruit growth, quality and yield is nitrogen (Rabe, 1994). In orchards where nitrogen level is below optimum, pre-blossom urea applications increased yield significantly (Rabe, 1994). Urea is usually applied as pre-blossom foliar fertilizer, as it is rapidly and efficiently absorbed by leaves and is cost effective. Foliar application of urea has been found to enhance fruit size, peel thickness, juice content, yield as well as total soluble solids (TSS) and acid content in some citrus species (Khan et al., 2009) although there are reports where nitrogen application resulted in lower fruit quality (Wutscher, 1997). Micronutrients sprays are also a common practice to rapidly satisfy plant needs (Swietlik, 2002; Tariq et al. 2007). Zinc is among the micronutrients used widely in citrus industry as foliar spray application; mainly due to its effect on normal plant development, as it is involved in the biosynthesis of tryptophan, a pre-cursor of the natural occurring auxin indole-3-acetic acid (Swietlik, 2002; Tariq et al., 2007).

Other agricultural practices which may increase yield and improve fruit quality are also the application of plant growth regulators, especially gibberellic acid at full bloom, to increase fruit set and the synthetic auxin triclopyr [3,5,6-trichloro-2-pyridyl)-oxyacetic acid] during fruitlet growth to increase fruit size (Agusti et al., 2002a; Chao and Lovatt, 2010).

The aim of the present trial was to study the effects of fertilizer application (zinc plus urea), of the synthetic auxin triclopyr and of girdling on total yield and fruit quality attributes of Nova mandarin trees.

Materials and Methods

Plant material – Site location

The experiment was conducted at Sagiada village, Thesprotia county, Greece (Longitude: 20° 11' 13" East, Latitude: 39° 37' 36" North, altitude: 50m). Trees were six years old "Nova" [(*Citrus clementina* Hort. Ex Tanaka x (*Citrus paradisi* Macf.x *Citrus tangerine* Hort. Ex Tanaka)] mandarins grafted on Swingle citrumelo rootstock, planted at 5 m x 4 m distances.

Treatments

Four treatments were applied, i.e. a) the control, b) two pre-bloom (15 and 5 days before full bloom (DBFB)) applications of zinc (as zinc sulfate at 1.2 g L^{-1}) combined with a pre- (5 DBFB) and a post-bloom (15 days after full bloom) application of urea (at a dose rate of 0.46 g L^{-1} each time) plus a soil application of ammonium nitrate (45 days after full bloom (DAFB) at a dose rate of 2 kg per tree, c) pre-bloom girlding (15 DBFB) and d) two spray applications of triclopyr with a 10 days interval, when the fruits had equatorial diameter of approximately 12-20 mm, using the commercial formulation Maxim (10% w/w triclopyr) TB at a dose rate of 10 mg L^{-1}. In all spray applications a non-ionic surfactant was used at a dose rate of 1mL L^{-1}. Applications were conducted till run-off. Girdling consisted of the removal of a 5 mm wide strip of bark without injuring the xylem in two out of the four scaffolds of the tree (Figure 1).

Figure 1. Healing of girdled zone approximately two months after girdling.

Sampling – Fruit physical characteristics – Juice pH, titratable acidity, total soluble solids

Healthy fruits without any symptoms of pest infestation or disease infection were sampled separately from each tree at fully ripe stage. At least thirty fruits per tree were sampled according to an equatorial pattern (East-West-North-South) from the periphery of the canopy of each tree, avoiding fruits situated at the top, bottom or deep inside the foliage. The sampled fruits were put into plastic bags and transferred to the laboratory within the same day, where they were processed immediately.

Sampled fruits were individually weighed, and the equatorial and polar diameters were measured with a digital caliper. Flesh and peel weights were determined and the peel thickness and number of seeds were measured. Fruit peel color was measured at three different points around the equatorial region of each fruit, using a Minolta CR 300 reflectance Chroma Meter (Minolta, Osaka, Japan) which provided CIE L*, a* and b* values. These values were used to calculate hue angle degree (h^o=arctan ($b^* a^{*-1}$)), where 0^o = red-purple; 90^o =yellow; 180^o = bluish-green; and 270^o = blue, and Chroma ($C^*=(a^{*2}+b^{*2})^{1/2}$), indicative of the intensity or color saturation and color index according to Singh and Reddy (2006). Fruits were then hand squeezed and the juice passed through a strainer. The juice was analyzed for total soluble solids (TSS), pH and titratable acidity (TA). TSS was evaluated at 20 C^o with an Atago 8469 hand-refractometer (Atago Co. Ltd, Tokyo, Japan) and expressed as ^{o}Brix. pH was measured after dilution of the juice at a ratio 1:20 with distilled water. TA was determined in the same diluted juice solution by titrating to pH 8.2 using 0.1 N NaOH and expressed as % (w/v) citric acid.

Phenolic compound determination – Antioxidant activity measurement

For phenolic compounds and antioxidant activity analysis the fresh squeezed juice was diluted 1:1 with methanol (Bronner and Beecher, 1995; Gattuso et al., 2007) and extracted at 40 ^{0}C with periodical agitation for 1h. The methanolic solution was then centrifuged at 4000g for 10min and the supernatant was stored at -25 C^o until analysis. For the rest of the analyses the supernatant juice was stored under the same conditions till analysis.

The methanolic fraction of the juice solution was assessed for total phenol, total o-diphenol and total flavonoid concentration according to Roussos et al. (2009) and the results expressed as mg equivalent tannic acid (TAE), caffeic acid (CAE) and caffeic acid (CAE) respectively.

The antioxidant capacity of the methanolic solution of the juice was evaluated using the diphenyl picryl hydrazyl (DPPH) and ferric reducing/antioxidant power (FRAP) assays (Klimczak et al., 2007). A small fraction of the methanolic solution was centrifuged at 4000g for 5 min and the supernatant juice was analysed for its antioxidant activity after being properly diluted.

Juice carbohydrate determination

Carbohydrate determination was performed by HPLC equipped with a refractive index detector in defrosted juice solution after proper dilution with HPLC grade water. A Waters 510 isocratic pump was used at a flow rate of 0.6 mL min^{-1} of water, while the separation of the carbohydrates was achieved through a Hamilton HC-75 cation exchange column, calcium form (Ca^{++}) (Hamilton, Bonaduz, Switzerland), thermostated at 80 ^oC. The sweetness index (SI) of the fruit, an estimate of the total sweetness perception, was calculated based on the relative amount and sweetness properties of each individual carbohydrate (Keutgen and Pawelzik, 2007).

Statistical analysis

The trial followed the completely randomized design with three replications of a single tree each. Data on the effect of the various treatments were analyzed as a One-Way Anova and differences were evaluated by Tukey HSD test at α=0.05.

Results and Discussion

Effect of treatments on yield and fruit physical characteristics

There were not any significant differences among treatments concerning yield (Table 1). Application of zinc plus nitrogen (as urea and ammonium nitrate) along with tryclopyr application resulted in significant fruit weight enhancement compared to control treatment (Table 1). This was not observed concerning fruit diameter and length or their ratio, which were similar between treatments. Zinc application in citrus species seems to have significant effects on shoot growth and fruit yield only under severe zinc deficiency (Boaretto et al., 2002; Swietlik, 2002), which was not the case in our study, based on observations made macroscopically. Nitrogen application though has been found to increase fruit set in citrus (Rabe, 1994) and to enhance fruit weight and size (Wutscher, 1997; Khan et al., 2009) as in the present trial, probably due a higher leaf to fruit ratio achieved by nitrogen application through enhancement of shoot growth and to a lower competition for nitrogen between growing fruits and shoots. Similar results concerning triclopyr treatment has been reported by Agusti et al. (2002b) working with Satsuma mandarin, while it increased yield of Nova mandarin too (Greenberg et al., 2006). It seems that triclopyr increases fruit weight probably due to an enhancement of fruit sink strength (Elotmani et al., 1993; Agusti et al., 2002b) and stimulation of cell expansion (Agusti et al., 1995). On the other hand girdling increased fruit weight and size but not significantly, in accordance with trials on Mihowase satsumas, where girdling did not have any effect on fruit size distribution (Peng and Rabe, 1996). This could be attributed to increased fruit set caused by girdling, which results in major competition for assimilates between growing fruits and thus similar fruit weight with control (Cohen, 1984).

Treatments did not have a significant effect on flesh weight, although the application of triclopyr resulted in lower flesh participation percentage per whole fruit compared to control. The opposite stood for peel weight and peel thickness, where triclopyr application increased their values, especially compared to control treatment. It seems that part of triclopyr effects on fruit weight enhancement is partly attributed to peel thickening. The same has been reported by Elotmani et al. (1993) who found an increase of peel weight in absolute value but not relatively to whole fruit weight after auxin treatment.

Juice volume was lower and seed number higher in fruits from control trees compared to all other treatments. This is in accordance with the findings reported by Elotmani et al. (1993) who found higher juice volume in fruits treated with auxin but opposite to the findings of Chao and Lovatt (2010) who found reduced juice volume after triclopyr application in “Fina Sodea” Clementine mandarin. This could be partly attributed to different species or cultivars as well as to different application time. According to Agusti et al. (2002a), the application of auxin at the onset of cell enlargement stage stimulates cell expansion of juice vesicles, increasing the vesicle capacity for juice accumulation. On the other hand, the effect of girdling on juice volume is rather inconsistent.

Table 1. Effects of the various treatments on yield and fruit physical characteristics.

Treatments	Yield per tree (kg)	Fruit weight (g)	Fruit diameter (mm)	Fruit length (mm)	Diameter/length	Flesh weight (g)	Flesh (%)	Skin weight (g)	Skin thickness (mm)	Juice (mL)	Seed number
Control	18.0 a	137.0 b	66.4 a	55.4 a	1.2 a	109.1 a	79.5 a	28.2 b	2.62 b	57.2 b	8.6 a
Zn/Urea/Ammonium nitrate	16.3 a	158.2 a	68.9 a	57.6 a	1.2 a	124.2 a	78.0 ab	35.5 a	3.02 b	63.4 a	4.6 b
Maxim	18.9 a	156.1 a	68.6 a	58.3 a	1.18 a	117.6 a	76.7 b	35.5 a	3.98 a	63.3 a	5.13 b
Girdling	16.5 a	144.3 ab	67.0 a	57.0 a	1.18 a	114.7 a	78.8 ab	30.5 ab	2.91 b	62.6 a	4.2 b

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD test at α=0.05.

According to Peng and Rabe (1996) girdling did not have any significant effect on juice volume, while Mostafa and Saleh (2006) report lower juice volume after girdling. Similar results have been reported after zinc or urea application, since Boaretto et al. (2002) and Tariq et al. (2007) did not find any significant effect of zinc application on juice volume of orange fruits, while the application of urea increased juice volume of kinnow mandarin (Khan et al., 2009) and decreased that of Valencia orange (Wutscher 1997). It seems that the response of the tree to foliar application of zinc and urea is quite complicated and depends on many factors other than the species and cultivars themselves.

Effect of treatments on juice characteristics

The titratable acidity, pH and maturity index (as the ratio of total soluble solids to titratable acidity) were not significantly affected by treatments (Table 2). This is in accordance with the findings of Agusti et al. (2002) on the effects of auxins on citrus fruit quality characteristics. Titratable acidity was slightly but not significantly higher in triclopyr treated fruits compared to control, which is in accordance with the findings of Chao and Lovatt (2010). Although girdling has been reported to induce a significant increase of total soluble solids (Peng and Rabe, 1996), the slightly higher fruit weight and juice volume of the fruits of girdled scaffolds in the present trial, could have functioned as a dilution agent, resulting in low total soluble solids of the juice. On the other hand the application of zinc and nitrogen resulted in lower total soluble solids than control, in compliance to Wutscher (1997) who found a significant decrease of total soluble solids of the juice after urea application in "Valencia" trees.

Table 2. Effects of the various treatments on juice quality characteristics.

Treatments	pH	TA (% citric acid)	TSS (^{o} Brix)	Maturity index
Control	3.39 a	1.17 a	11.5 a	10.1 a
Zn/Urea/Ammonium nitrate	3.56 a	0.99 a	9.47 b	9.62 a
Maxim	3.45 a	1.23 a	10.6 ab	8.68 a
Girdling	3.37 a	1.21 a	10.8 ab	9.2 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD test at α=0.05.

Effect of treatments on fruit color

Treatments had a significant effect on all fruit color parameters (Table 3). Girdled scaffolds produced fruits with lower values of a and higher b, L and Hue values than triclopyr treated ones, but lower color index value than the other treatments. Application of zinc and nitrogen resulted in fruits with lower Chroma values than the other treatments. Generally, girlding has been found to advance color development in citrus (Peng and Rabe, 1996) through the high accumulation of assimilates above the girdling zone, while application of urea seems to reduce peel coloring (Wutscher 1997).

Table 3. Effects of the various treatments on peel color parameters.

Treatments	a	b	L	Hue	Chroma	Color index
Control	33.6 ab	69.0 ab	64.6 b	64.0 bc	76.8 a	7.54 a
Zn/Urea/Ammonium nitrate	31.8 b	66.9 b	63.7 b	64.6 ab	74.0 b	7.46 a
Maxim	34.7 a	67.0 b	63.6 b	62.6 c	75.4 ab	8.15 a
Girdling	31.2 b	70.2 a	66.3 a	66.1 a	76.8 a	6.70 b

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD test at α=0.05.

Effect of treatments on juice carbohydrate concentration

Girdling resulted in fruits with higher concentration of sucrose and glucose than the other treatments, and higher concentration of fructose compared to triclopyr and zinc plus urea application (Table 4). As a consequence of the higher individual carbohydrate concentration, fruits of girdled scaffolds had higher total sugars concentration than all other treatments, followed by those fruits produced under control treatment and lastly by fruits produced under triclopyr and zinc plus nitrogen application. Sweetness index was also higher in fruits produced in girdled scaffolds compared to all other treatments. The best known effects of girdling are presumably the accumulation of assimilates above girdle, due to the blocking the downward translocation of soluble sugars, altering thus the carbohydrate partitioning (Li et al., 2003; Rivas et al., 2006, 2007) and increasing the availability of carbohydrates to the fruit, inducing higher sugar concentrations (Agusti et al., 2002). According to Agusti et al. (2002) the application of triclopyr at the cell enlargement stage increases the hexose concentration in the fruit, due to the promotion of sink strength. In the present experiment triclopyr application reduced total sugar concentration of the juice. This discrepancy could be attributed to the different cultivars used between experiments as well as to the second triclopyr application of the present experiment, compared to the single application of Agusti et al. (2002) trials. On the other hand, the higher juice volume achieved by triclopyr treatment than control in the present trial could have resulted in significant dilution of sugars in the juice, justifying the present results.

Table 4. Effects of the various treatments on juice carbohydrates' concentration (g 100 mL^{-1}).

Treatments	Sucrose	Glucose	Fructose	Total sugars	Glucose: Fructose	Sweetness index
Control	5.17 b	1.2 b	1.55 ab	7.92 b	0.783 a	11.5 b
Zn/Urea/Ammonium nitrate	4.34 b	0.99 b	1.34 b	6.67 c	0.757 a	9.74 c
Maxim	4.52 b	1.15 b	1.31 b	6.98 c	0.863 a	10.0 c
Girdling	6.32 a	1.54 a	1.66 a	9.52 a	0.89 a	13.6 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD test at α=0.05.

Effect of treatments on phenolic compounds

Fruits from girdled scaffolds exhibited higher values of total phenols and flavonoids than fruits from all other treatments (Table 5). o-diphenol concentration was higher in fruits from girdled scaffolds compared to control, but without any significant difference with those produced after nutrient and triclopyr application. Similar results have been reported by Kubota et al. (1993) on peaches and by Khandaker et al. (2011) working with *Syzygium samarangense,* who all attributed this increase to the enhancement of phenylalanine ammonia lyase activity (the key enzyme in phenolic compounds biosynthesis).

Table 5. Effects of the various treatments on the total phenols, total o-diphenols and total flavonoids' concentration and antioxidant capacity of the juice.

Treatments	Total phenols (TAE) (mg L^{-1})	o-diphenols (CAE) (mg L^{-1})	Flavonoids (CAE) (mg L^{-1})	DPPH (%)	FRAP (μm Trolox equiv)
Control	34.2 b	1.38 b	2.2 bc	21.2 a	1.86 ab
Zn/Urea/Ammonium nitrate	29.7 b	1.54 ab	1.9 c	20.1 a	1.59 bc
Maxim	30.2 b	1.56 ab	2.49 b	19.7 a	1.36 c
Girdling	40.6 a	1.93 a	3.09 a	20.5 a	2.18 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD test at α=0.05.

Abbreviations: TAE, tannic acid equivalents; CAE, caffeic acid equivalents.

Antioxidant capacity of the juice measured by the DPPH assay did not exhibit any significant difference between treatments. On the other hand, when the antioxidant capacity was measured by FRAP assay, fruits produced under girdling treatment exhibited the highest values with significant difference from those fruits produced under nutrient and triclopyr application. Unfortunately there are not much data on the effect of girdling on phenolic compound concentration. The accumulation of phenolic compounds in fruits from girdled scaffolds could be justified based on the hypothesis of growth differentiation balance (Kuokkanen et al., 2001). According to this hypothesis, only when carbohydrate biosynthesis overlap the demands for growth the excess carbon can be used for carbon-based secondary metabolites such as phenolic compounds (Kuokkanen et al., 2001). As girdling is assumed to enhance carbohydrate accumulation above girdle zone, the excess carbohydrates could have been used to the biosynthesis of phenolic compounds, which are known antioxidants, enhancing thus the antioxidant capacity of the juice produced from fruits of girdled scaffolds.

Conclusion

The application of urea plus zinc, triclopyr and the execution of girdling at the crop growth stages described earlier could be a valuable tool in improving "Nova" mandarin fruit quality, based on both physical and biochemical quality characteristics. Girdling improved significantly the quality characteristics of the fruits, enhancing the sweetness index, as a result of increased carbohydrate concentration, and increasing the phenolic compound concentration of the juice.

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