Effect of Gibberellic Acid, Urea and KNO_3 on Yield and on Composition and Nutritional Quality of Clementine Mandarin Fruit Juice

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Abstract

Clementine mandarin is an alternate-bearing cultivar with fruit of small to medium size, easy peeling, juicy and with an excellent eating quality. It has been reported that preharvest as well as postharvest factors (such as cultural practices and storage conditions) affect yields and composition and quality of citrus fruit. This study reports on research carried out for 2 consecutive years (an "off year" with yield ≈ 40 kg/tree and an "on year" with yield ≈ 140 kg/tree for untreated control trees) to assess the influence of foliar sprays - of i) urea (containing 46%N) at 1 and 1.6% (w/v) at flower initiation-differentiation to increase flowering, ii) gibberellic acid (GA_3, 10 ppm) applied during flowering to increase fruit set and iii) KNO_3 (at 5% w/v) applied at 'June drop' to increase fruit size - on juice quality of 'Cadoux' clementine fruit. Mature trees were used and treatments were applied as foliar sprays to the point of run-off. For the "on year" fruit, juice content of mature fruit was not affected by treatment, nor was the maturity index or NO_3^- content of the juice. Free aminoacid content was lowest in fruit of untreated trees and in fruit of urea treated trees and greatest in fruit of GA_3 and KNO_3 treated trees whereas NH_4^+ content had the opposite trend. Ascorbic acid content was greatest in fruit from trees treated with urea at 1.6%, GA_3 and KNO_3. In the "off year", urea increased juice content, reduced acidity and increased the maturity index. In storage, juice acidity decreased and, consequently, the maturity index was increased with the greatest values recorded for the urea treatment. Significance of these results in respect to nutritional value of the fruit is discussed.

INTRODUCTION

Clementine mandarin (*Citrus reticultata* Blanco) produces fruit of an excellent eating quality. The consumer pays premium prices for this easy peeling fruit particularly when it is seedless, adequate in size, good tasting, and deep orange in rind color. In terms of production, it has an alternate-bearing pattern with a large number of fruit, typically of small size and of low commercial value, in an "on" year followed by an "off" year with a few fruit of large size, with no commercial value. Low yield in the "off" year is due to low flower intensity.

In mandarins, the highly desirable trait of seedlessness is associated almost universally with the problem of reduced fruit set and inability to regularly produce a commercially harvestable crop on an annual basis and/or with the problem of small-sized fruit (Hodgson, 1967; Davies and Albrigo, 1994). Use of gibberellic acid during full bloom-to-petal fall as a foliar spray increased fruit set significantly (El-Otmani et al.,

1992) and this is a common practice in most clementine-producing countries around the world (El-Otmani et al., 2000a).

In addition, we have recently reported that in two clementine selections urea applied during the winter (i.e. during the period of flower initiation-differentiation) enhanced flowering (El-Otmani et al., 1998a, 1998b) and increased fruit set and size (El-Otmani et al., 2000a).

Moreover, DuPlessis and Koen (1988) reported that 2 foliar sprays of KNO_3 (at 4%) applied in November and December (corresponding to the end of the "June drop" period increased fruit size in Valencia 'orange'. Erner et al. (1993) also reported that a mixture of KNO_3 (at 5%) + 2,4-D (at 20 ppm) applied to the tree at the end of May to beginning of June (i.e. 6-8 weeks past petal fall) also increased fruit size of 'Valencia', navels and grapefruit. These treatments are acceptable in years of high yields.

Production of greater yields but at a regular basis, associated with enhanced fruit quality and better nutritional value should bring greater revenue to the grower. The objective of this research is to investigate effect, on juice quality, of i) urea applied at flower-initiation-differentiation to increase flowering and fruit number at harvest, ii) gibberellic acid applied at flowering to increase fruit set, and iii) KNO_3 applied at 'June drop' to increase fruit size. In particular, besides the commonly analysed parameters (juice acidity and total soluble solids), vitamin C (ascorbic acid) is an important quality component of citrus because it is essential in the human diet as it prevents the common cold and is an important free radical scavenger in the human diet (MacKersie and Lesham, 1994) and it is believed to reduce cancer and cardiovascular disease (Simon, 1992). Furthermore, there is a growing concern among consumers regarding the levels of nitrogenous compounds in their diets, particularly nitrates. Although, there are no standard limits set for fruits and vegetable juices and despite the fact that the published acceptable nitrate levels in drinking water (i.e. 50 mg/liter) is strongly criticized today (Apfelbaum, 2001), this research investigates effect of the treatments (particularly urea and KNO_3) on these compounds and on total aminoacids in clementine mandarin fruit juice.

MATERIALS AND METHODS

Plant Material and Tree Growing Conditions

The experimental orchard used in this study was located in the Souss Valley of Southern Morocco, characterized by a semi-arid climate with low rainfall (≈ 200 mm/year) and temperatures ranging from +5°C in winter to +40°C in summer. Trees of 'Cadoux' clementine (*Citrus reticulata* Blanco) grafted on sour orange rootstock (*Citrus aurantium* L.), planted in 1969 at a spacing of 6 m x 6 m on a silty-clay soil with a pH ≈ 8.3 were used. Cultural practices are considered optimal for the region. Water is applied as needed using microsprinkler irrigation, and fertilization is essentially through fertigation using ≈ 180 to 220 kg of N 60 kg P_2O_5 and 180 kg K_2O per hectare per year.

Micronutrients, particularly Fe, Mn and Zn are applied, as needed, in the spring. In addition, for fruit setting the trees also regularly received 2 gibberellic acid (GA_3 at 10 ppm) treatments, one at full bloom and the other at beginning of petal fall.

Treatment Application and Experimental Design

In addition to the routine cultural practices described above, the treatments were applied in microdroplets as foliar spray to whole trees using a gun sprayer to dispense ≈ 10 l/tree. The spray mix also contained a non-ionic wetting agent (Agral containing 900 g of alkyl phenol ethylene oxyde per l and used at 30 ml/100l).

The compounds tested were: Commercially available urea (containing 46% N) tested at the rates of 1 and 1.6 kg/100l and applied at flower initiation-differentiation (2 January for 1998 and 28 January for 1999). GA_3 at 10 ppm at white flower bud stage (4 March for 1998 and 2 March for 1999). KNO_3 applied at 5 kg/100l at "June drop" (4 May for 1998 and 8 June for 1999).

A completely randomised design was adopted with six two-tree replications per treatment.

Yield, Fruit Sampling, Storage and Analyses

Fruit was harvested at maturity and yield in kg and in number of fruit per tree was obtained. One batch of samples consisting of 10 fruits per replication, was analyzed immediately after harvest. Only sound fruit with a diameter of 55-64 mm were used. A second batch of samples consisting of 15 fruits/replication was stored at 4°C ± 1°C and 85-95% relative humidity. For each replication, ten of these fruits were then used for analysis after 30 days in storage.

These fruits were used for the determination of juice content gravimetrically. A portion of the juice was then used to determine total soluble solids by a refractometer and titratable acidity by NaOH (0.1N) titration. The other portion was used to determine ascorbic acid content using the HPLC method developed by Lees and Coates (1987) and juice ammonia and nitrate contents using the distillation method of Keeney and Nelson (1982). In addition, effect of the treatments on total aminoacids in the juice was determined using the formol index (AFNOR, 1982).

Leaf Sampling and Analyses

Leaf sampling and analyses were performed in 1998 according to the methods of Chapman and Pratt (1978). In short, leaves of 6 to 7 months of age were sampled from non fruiting terminals of the previous spring flush. Samples consisted of 20 leaves each, randomly sampled to include all 4 tree quadrants. Nitrogen was analysed using the Kjeldahl method, P by colorimetric determination and K by spectrophotometry.

Data Analyses

An analysis of variance was performed on the data and means were separated using Newman and Keuls method at the 5% level.

RESULTS

Yield and Leaf Mineral Content

Yield was significantly increased by all of the treatments for the “off year” (i.e. 1998) and the “on year” (i.e. 1999) and the cumulative yield was greatest for the GA_3 treatment applied at the white flower bud stage followed by the urea at 1.6% applied at flower initiation differentiation (Table 1). The increase was mainly due to increased fruit number at harvest and, in some cases, to increased fruit size particularly during the “off year”.

For control untreated trees, leaf nitrogen content was close to optimum for the region (Lekchiri, 1996) but urea and GA_3 increased it significantly (Table 2). Leaf P and K levels were not statistically affected by the treatments although there appears to be some increase in leaf K. It is noteworthy that leaf P was slightly lower and leaf K was in the optimum range for clementine mandarin grown in Morocco (Lekchiri, 1996).

Fruit Juice Organoleptic Quality at Harvest and in Storage

During the “off year”, at harvest, fruit juice content was greater in fruits from urea treated trees (Table 3). These fruits also had lower acidity (TA) and greater total soluble solids (TSS) compared to fruits from control untreated trees. GA_3 applied at flowering and KNO_3 applied during the “June drop” period had greater (but generally lower than the values obtained for urea) maturity index (i.e. TSS/TA ratio) compared to fruit from untreated trees. After a 30 day storage period, fruit juice content did not change except for the GA_3 treatment for which there was a slight decrease (Table 3). In addition, for all treatments, acidity decreased and consequently, the ratio TSS/TA increased by one to two units over the 30-day storage and the final values were greatest for the urea treatment and lowest for fruit of the untreated trees.

For the "on year" fruit, juice content was not affected by any of the treatments, nor was the ratio TSS/TA (Table 4). However, fruit from trees that received GA_3 and KNO_3 had the greatest values of juice titratable acidity.

Ascorbic acid content increased by application of urea at 1.6%, GA_3 and KNO_3 but was not affected by urea applied at 1% (Table 4).

Fruit Juice Ascorbic Acid, Formol Index, and Ammonium and Nitrate Levels

Juice aminoacid content (as indicated by the formol index) was significantly reduced in fruit from trees treated with GA_3 and KNO_3 and somewhat reduced in fruit from trees receiving urea at 1.6% (Table 4) corresponding to fruit from trees that gave the greatest fruit yields and particularly the greatest fruit numbers (Table 1). Conversely, juice ammonium content increased in fruit from trees treated with GA_3 and KNO_3 with intermediate values for fruit from trees receiving urea at 1.6% (Table 4). Nitrate content, however, was not affected by any of the treatments (Table 4).

DISCUSSION

Urea applied prebloom was reported to increase flower initiation and thus increase flower intensity of the clementine mandarin (El-Otmani et al., 1998a; 1998b; 2000b) which is again confirmed in this study and was also reported to increase fruit set and size (Ali and Lovatt, 1994; El-Otmani et al. 2000a). Leaf N levels were significantly increased as a result of this treatment. We also reported that urea increased leaf area and dry matter content (El-Otmani et al., 2002). Therefore, because growing fruit are strong sinks, it can be assumed there is an increase in the synthesis of macromolecules and their mobilization from the leaf to the fruit thus increasing fruit set and size.

GA_3 is known to increase fruit set when applied during the period of full bloom-to-petal fall (see the review by El-Otmani et al., 2000a) but, to our knowledge, this is the first report on it having a similar effect when used prior to this stage (Table 1). Other researchers have reported that low fruit set in cultivars that set a few fruit-to-none in the absence of cross-pollinisation was linked to low gibberellin content in the developing ovaries (Talón et al., 1992). In addition, gibberellin was shown to increase sink strength in ovaries and enhance assimilate export to the developing fruit (Mauk et al., 1986). García-Martínez and García-Papí (1979) reported data suggesting that foliar-applied GA_3 increased translocation of minerals from leaves to the growing fruit. However, because our leaf analysis data show that GA_3-treated leaves have mineral levels equal to (for P and K) or greater than (for N) those found in leaves of untreated trees, this indicates that if mineral translocation from leaves to fruits takes place and the levels are not depleted, it can be assumed that there is greater uptake by the roots or mobilisation from other tissues such as the bark. It can then be hypothesized that this phenomenon had an effect on fruit set through reduced competition for the nitrogenous compounds available for fruit set and growth, which then lead to an increased yield.

Increased yield and fruit size as a result of KNO_3 foliar spray were reported by Bar-Akiva (1963) on 'Valencia' orange. However, it is not clear whether in our study this treatment increased leaf K since the leaves were not analysed; however, leaf K of the untreated control trees were in the optimum range (Lekchiri, 1996). The great increase in fruit number due to KNO_3 (Table 1) particularly during the "on year" (i.e. 1999) warrants further investigation. This increase has reduced fruit size because the increase recorded for yield in kg was much smaller than that for yield in fruit number (Table 1). Potassium plays an important role in tree physiology and growth but its effect on reducing fruit drop is noteworthy.

Juice quality was significantly affected by the year and by the treatments with an increase of the maturity index during storage. The fact that urea reduced total soluble solids during an "on year" (Table 4) agrees with the results reported by Govind and Pasadesh (1986) indicating a depressive effect of nitrogen on sugar accumulation.

However, during the "off year" fruit from urea-treated trees accumulated more sugars than untreated trees (Table 3).

Ascorbic acid content was in the same magnitude as that reported for pasteurised Moroccan orange juice (Kaanane et al., 1988) but its enhancement by some of the treatments (Table 4) used in these experiments warrants further consideration as this might be an important way for enhancing the nutritional value of clementine.

Fruit nitrate content was generally lower than 20 mg/l (Table 4), which is much lower than the generally accepted 50 mg/l as the maximum value for drinking water (Apfelbaum, 2001) but the significance of this is unknown at this time. The formol index values obtained were also in the same range as those reported by Kaanane et al. (1988). These values did not change with urea treatment but were reduced with GA_3 and KNO_3 and the trend was exactly the opposite for ammonium indicating a negative correlation between these two nitrogenous compounds. This negative correlation was also obtained in another study where we showed a decrease in the formol index and an increase in ammonium content with increasing fruit size for 'Ortanique' mandarin fruit (El-Hassainate, 2000).

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Tables

Table 1. Effect of foliar application of urea at flower initiation-differentiation, of GA_3 at flowering and of KNO_3 during the "June drop" period on yield and fruit size for 'Cadoux' clementine mandarin in 2 consecutive years (1998 and 1999).

Treatment	Yield					Fruit weight (g/fruit)	
	kg/tree			Number of fruit/tree			
	1998	1999	Cumulative	1998	1999	1998	1999
Untreated control	40.9c[z]	136.2c	177.1	735b	2313c	55.6b	58.8a
Urea (1% w/v) at flower initiation-differentiation	63.1ab	155.8bc	218.9	1051a	2573c	60.0a	60.6a
Urea (1.6% w/v) at flower initiation-differentiation	59.0b	204.2a	263.2	937a	3421ab	63.0a	59.7a
GA_3 (10 ppm) at white flower-bud stage	68.2a	221.7a	289.9	1101a	3793a	61.9a	58.4a
KNO_3 (5%) at "June drop"	63.0ab	168.1bc	231.1	1027a	3156b	61.3a	53.3b
Significance level [y]	***	***	-	**	***	***	*

[z] Within columns, means followed by the same letter are not significantly different at 5% level (Newman-Keuls test)
[y] Differences are significant at 5% (*), 1% (**) or 0.1% (***) level

Tables 2. Effect of application of urea at flower initiation-differentiation and of GA_3 at flowering on leaf N, P and K contents (in % dry matter) of 'Cadoux' clementine mandarin.

Treatment	Mineral content		
	N	P	K
Untreated control	2.07b[z]	0.09	1.29
Urea (1% w/v)	2.42a	0.10	1.45
GA_3 (10 ppm)	2.52a	0.09	1.33
Significance level [y]	**	NS	NS

[z] Within columns, means followed by the same letter are not significantly different at 5% level (Newman-Keuls test)
[y] Differences are not significant (NS) or significant at 1% (**) level

Table 3. Effect of foliar application of urea at flower initiation-differentiation, of GA_3 at flowering and of KNO_3 during the "June drop" period on fruit juice organoleptic quality at harvest (on 10 Nov. 1998) and after 30 days in storage for 'Cadoux' clementine mandarin. Data are for the 1998 year ("off year")

Treatment	Juice content (%)		Titratable acidity (TA; %)		Total soluble solids (TSS ; °Brix)		Ratio TSS/TA	
	At harvest	After storage	At harvest	After storage	At harvest	After storage	At harvest	After storage
Untreated control	50.8b[z]	51.2a	1.22a	1.00a	9.1c	9.6c	7.5c	9.6c
Urea (1% w/v) at flower initiation-differentiation (21 janv. 1998)	54.2a	51.7a	1.00b	0.92b	10.5b	11.1a	10.6a	12.1a
Urea (1.6% w/v) at flower initiation-differentiation (21 jan. 1998)	53.9a	53.0a	0.98b	0.90b	10.3b	10.1b	10.5a	11.7a
GA_3 (10 ppm) at white flower-bud stage (4 mar. 1998)	51.3b	47.4b	1.16a	0.90b	11.8a	10.4b	10.1ab	11.5ab
KNO_3 (5%) at "June drop" (4 May 1998)	51.6b	51.4a	1.03b	0.96a	9.7bc	10.5b	9.5b	11.0b
Significance level[y]	*	*	**	*	**	**-	***	**

[z] Within columns, means followed by the same letter are not significantly different at 5% level (Newman-Keuls test)
[y] Differences are significant at 5% (*), 1% (**) or 0.1% (***) level

Table 4. Effect of application of urea at flower initiation-differentiation, of GA_3 at flowering and of KNO_3 during the "June drop" period on fruit juice organoleptic quality, ascorbic acid content, formol index, ammonium and nitrate levels at harvest (23 Dec. 1999) for 'Cadoux' clementine mandarin. Data are for the 1999 year ("on year")

Treatment	Juice content (%)	Titratable acidity (TA; %)	Total soluble solids (TSS; °Brix)	Ratio TSS/TA	Ascorbic acid content (mg/100 ml)	Formol index	Ammonium content (mg/l)	Nitrate content (mg/l)
Untreated control	48.5	0.69b[z]	11.3b	16.3	43.0b	23.8a	17.5b	15.5
Urea (1% w/v) at flower initiation-differentiation	54.0	0.62b	10.1d	16.3	41.6b	23.7a	17.8b	19.4
Urea (1.6% w/v) at flower initiation-differentiation	48.1	0.66b	10.6c	16.1	56.4a	21.6ab	20.1ab	17.6
GA_3 (10 ppm) at white flower-bud stage	49.5	0.82a	12.3a	15.0	56.3a	20.2b	21.1a	15.5
KNO_3 at "June drop"	51.1	0.73b	11.1b	15.2	56.3a	20.2b	21.1a	15.5
Significance level[y]	NS	**	***	NS	***	**	*	NS

[z] Within columns, means followed by the same letter are not significantly different at 5% level (Newman-Keuls test)
[y] Differences are not significant (NS) or are significant at 5% (*) , 1% (**) or 0.1% (***) level