Proc. Int. Soc. Citriculture, 2004. 479-483.

The Potential Use of GA_3 and Urea to Manipulate Flowering and Reduce the Alternate-Bearing Pattern of the 'Nour' Clementine Mandarin

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Additional index words. Citrus reticulata Blanco, flowering induction, flowering inhibition, alternate-bearing index, yield

Abstract. **The 'Nour' Clementine (*Citrus reticulata* Blanco cv. Nour) is a very important cultivar for the Moroccan citrus industry. The fruit has adequate size, stores well, has an excellent eating quality and, above all, it is harvested late (December-January) compared to the other available Clementine selections. However, its production alternates significantly from year to year. Generally, in one year (i.e., 'ON year') it flowers abundantly with no or few vegetative shoots leading to a large crop; the following year (i.e., 'OFF year') trees of this variety generate mostly vegetative shoots and a few numbers of flowers leading to a few big fruits with rough greenish and thick rind. The aim of this study is to investigate the effect of use, during flower initiation-differentiation, of GA_3 (a flowering inhibitor) when an 'Off year' is expected and urea (a flowering inducer) when an 'OFF year' is expected, to reduce alternate bearing of this cultivar. One set of eleven-year old 'Nour' clementine trees was treated with GA_3 during three consecutive years (2001, 2002 and 2003) at two different concentrations (25 and 50 ppm) and at three different dates comprising the flower initiation/differentiation stage. Another group of trees was treated in 2001 and 2003 ('ON years') with GA_3 as described above and with urea (1% w/v) in 2002 ('OFF year') at the same physiological stage. Results show that the number of flowers was reduced by up to 83% in trees receiving GA_3; conversely, the number of vegetative shoots was greater in all GA_3-treated trees compared to the untreated controls. GA_3 alone did not reduce alternate-bearing of the trees; however, using GA_3 in the 'ON year' followed by urea in the 'OFF year' reduced the alternate bearing index.**

Introduction

Alternate bearing is an extreme form of irregularity of cropping; it can either be a physiological characteristic of the cultivar or related to climatic conditions, cultural practices or chemical treatments (Hoad, 1984). Biennialism is the tendency of a fruit tree to produce a heavy crop in one year ('ON year') followed by a light crop or no crop ('OFF year'). The phenomenon is widespread and occurs in both deciduous and evergreen trees (Monselise and Goldschmidt, 1982).

Alternate-bearing is a major problem in citrus production all over the world, especially in easy peeling citrus such as clementine and mandarin varieties (Wheaton, 1992) and many marketing problems result from it. In the 'ON year' trees produce a large number of small size fruit and in the 'OFF year' trees produce a small number of large, rough and unattractive fruit (Monselise et al., 1981). This phenomen in citrus is known to be due to a lack of flowering in the spring following a heavy on-crop year, rather than to poor fruit set (Goldschmidt and Golomb, 1982). In addition, floral intensity (number of flowers) and yield and, therefore, the severity of alternate-bearing, is inversely proportional to the size of the preceding year's crop load (number of fruit) (Moss, 1971, 1973).

In Morocco, this phenomenon is common in clementines and the 'Nour' selection is an example. In fact trees of 'Nour' may produce over 200 Kg of fruit in one year and almost no fruit the following year.

One approach to control alternate-bearing is to manipulate flowering using the available easy-to-use techniques such as growth regulators and nutritional substances.

In fact, gibberellic acid (GA_3) is wildely used in citrus all over the world but for various objectives (see El-Otmani et al. (2000) and Benhamou (2003) and the references therein). Its inhibitory effect on flowering is well documented (Monselise and Halevy, 1964; Goldschmidt and Monselise, 1972; Moss et al., 1974; Guardiola, 1981). In addition to its inhibitory effect on flower induction, GA_3 foliar application during the flower initiation/differentiation stage increased the proportion of leafy generative shoots and vegetative shoots (Moss et al., 1977). Leafy inflorescences produce fruits with large size (Deidda and Agabbio, 1977), and vegetative shoots take a part in the accumulation of reserves for the current season fruiting and for the flowering process the following season (Goldschmidt and Golomb, 1982). In addition, because citrus flowers mostly on wood less than one year old, large vegetative shoot numbers constitute a large surface for flower production the following season. Also, many authors have reported that using GA_3 during the rest period of citrus trees can reduce the intensity of alternate-bearing [Moss et al. (1977) in 'Valencia' late; Guardiola et al. (1977) in 'Washington' navel and 'Navelate'].

Furthermore, the enhancing effect of nitrogen compounds (polyamines and their precursors, ammonia, urea…) on flowering and fruiting was show by several authors. Lovatt et al. (1988) reported that, under stress conditions', flowering was stimulated in stressed trees. Ali and Lovatt (1995) demonstrated that stress conditions increase the concentration of ammonia (NH_3-NH_4^+) in 'Washington' navel leaves and that there is a positive correlation between the concentration of ammonia in leaves during flower induction stage and the total number of generated flowers. In addition, they reported that winter foliar application of urea (source of nitrogen and precursors of polyamines) increased the number of generative shoots.

Thus, the aim of this study is to evaluate: 1) the effect of foliar application of GA_3 during flower initiation/differentiation stage for three consecutive years on flowering, vegetative growth and yield of the 'Nour' Clementine; 2) the effect of application of GA_3 during flower initiation/differentiation stage of the 'ON year' followed with application of urea at the same period but of the following 'OFF year'; 3) compare the two strategies as to their effect on the alternate-bearing index and cumulative yield.

Materials and methods

Plant material and treatments used

The study took place in a commercial grove in the region of Taroudant of southern Morocco.

Ten 'Nour' clementine (*Citrus reticulata* Blanco) trees grafted on sour orange (*Citrus aurantium* L.), and planted in 1990 at a spacing of 6mx4m were used. They were homogenous in vigor and showed no disease or deficiency symptoms. They were under a production program known to be optimal for the area.

In the first year of the study (2001; 'ON year') except for control untreated trees all the trees included in the experiment received a foliar application of GA_3 (used at 25 and 50 ppm) at one of three dates comprising the flower initiation/differentiation stage: Date 1: 08-01-2001, Date 2: 18-01-2001 and Date 3: 31-01-2001. In the following year (2002; 'OFF year') half of the trees of each combination date x concentration of GA_3 received GA_3 like in 2001 at one of the following dates: Date 1: 09-01-2002, Date 2: 19-01-2002 and Date 3: 29-01-2002; and the other half received at these same dates a urea treatment (containing 46% N) at 1% w/v as a foliar spray using approximately 10L / tree. In 2003, the trees received GA_3 at 25 and 50 ppm as in 2001 at: Date 1: 16-01-2003, Date 2: 29-01-2003 and Date 3: 07-02 -2003. Untreated trees were used as a control trees.

Parameters observed:

On each tree, 4 shoots composed of growth flush of the previous spring which also bore only one shoot of the growth flush of the summer were tagged in the winter after treatment application. These shoots were taken at 1.5 m height and from the various sides of the canopy. Their node numbers were counted and recorded. In the spring, the number of vegetative shoots, the number of inflorescences and the number of flowers were recorded for each node position. These counts were made at 2-week intervals and for the duration of the flowering period to obtain the date of full bloom.

At harvest, yield was obtained for each tree as fruit number and Kg per tree, and average weight for individual fruit was calculated. Because fruit yield was very small in 2002, individual fruit number was not obtained as it was nil in some trees.

Alternate-bearing index (I, %) was calculated using the formula of Hoblyn et al. (1936):

$$I (\%) = [1/N-1] [(a_1-a_2/a_1+a_2) + (a_2-a_3/a_2+a_3) + \dots + (a_{(n-1)} - a_n/a_{(n-1)}+a_n)] x100$$

With N: number of experiment years; n: index of the year of the observation; a: a_1, a_2, a_3, ... $a_{(n-1)}$, a_n: yiels of experiment years (signs of terms between brakets are ignored)

Because the duration of our study is three years, the formula will become:

$$I(\%) = 0.5 [(|a_1-a_2|/a_1+a_2) + |a_2-a_3|/a_2+a_3)] x 100$$

With a_1: yield in 2001; a_2: yield in 2002; a_3: and yield in 2003

Statistical analysis

A completely randomized design was used with 10 replications (one replication = one tree).

Data were subjected to an analysis of variance using STATITCF package. Mean separation was performed using Newman and Keuls test at α = 0.05%. Means are reported with their confidence intervals.

Results and discussion

Flowering and vegetative growth response to winter application of GA_3

Because the 'Nour' clmementine produced significantly more flowers on summer growth than on spring growth (data not shown) we report GA_3 effects on flowers borne on summer shoots only. In 2001 (i.e., 'ON year"), trees treated with 50 ppm GA_3 showed a great reduction in their flower number (46.2 to 71.5% reduction compared to untreated control, (Table 1)), but vegetative growth was greater in all GA_3-treated trees in comparison with untreated control trees which produced no vegetative shoots that year (Table 2). In 2002 (i.e., 'OFF year'), the number of flowers was the same for all of the treatments (including the untreated control) (Table 1) but the vegetative growth was greater for GA_3-treated trees regardless of timing and concentration of the application (Table 2). In 2003 (i.e., 'ON year'), GA_3 at 25 ppm reduced flowering by an average of 40% whereas the 50 ppm concentration reduced it by about 80% in comparison with untreated control trees (Table 1). However, vegetative growth was greater in GA_3-treated trees than in untreated ones. These results confirm those reported by Harty and Sutton (1992) in that when GA_3 was applied during flower initiation/differentiation period of 'Miyagawa' Sarsuma flower intensity was reduced by 52% to 63% with 50 ppm of GA_3 and number of generated vegetative shoots was increased compared to untreated controls.

Effect of GA_3 on yield, alternate-bearing index, total yield, number of fruit per tree and individual fruit weight (g/fruit)

In the years 2001 and 2002, yield was not affected by GA_3 treatment (Table 3) and neither was the number of fruit per tree and the individual fruit weight in 2001(Table 4). It is note-worthy that in 2002, trees yielded few large sized and rough and non-marketable fruit with no difference between treatments (data not shown). Conversely, in 2003, theses parameters were influenced by GA_3. GA_3 at 25 ppm in Date 1 and Date 3 gave the greatest yield (Table 3) because they generated the greatest number of fruit that were of large size (Table 4). But, the individual fruit weight was promoted by the application of 25 ppm of GA_3 regardless of the timing of the application. The difference between theses treatments can be explain by two hypotheses: 1) the use of GA_3 had probably overcome the problem of low fruit set or the excessive fruitlets fall by the promotion of vegetative growth which is the source of carbohydrates and hormones necessary for fruit set and growth, or 2) by increasing the percentage of leafy inflorescences (Deidda and Agabbio, 1977).

Table 1. Effect of concentration and timing of foliar application of GA_3 on the flower number/100 nodes formed on summer shoots for three consecutive years (2001 to 2003).

Treatments		Dates of full bloom according to year of study		
		21-03-2001	27-03-2002	26-03-2003
Untreated control		188.1 ± 41.7[Y]	11.9 ± 9.9	143.1 ± 78.1
GA_3 at 25 ppm	Date 1	147.9 ± 119.0	2.50 ±7.2	125.7 ± 47.0
	Date 2	168.9 ± 78.6	14.4 ± 20.0	38.0 ± 76.1
	Date 3	176.2 ± 88.4	2.9 ± 2.8	102.1 ± 95.4
GA_3 at 50 ppm	Date 1	53.6 ± 42.8	16.2 ± 13.6	24.2 ± 35.4
	Date 2	98.7 ± 69.2	3.4 ± 3.8	51.8 ± 48.1
	Date 3	101.6 ± 83.7	9.9 ± 8.8	37.6 ± 12.4
Significance level:	'date'	NS	NS	NS
	'concentration'	0.001	NS	0.001
	'interaction'	NS	0.05	NS

[Y]mean ± confidence interval

Table 2. Effect of the concentration and the timing of foliar application of GA_3 on the vegetative shoot number/100 nodes formed on summer shoots of the previous season for three consecutive years.

Treatments		Dates of full bloom according to year of study		
		21-03-2001	27-03-2002	26-03-2003
Untreated control		0 ± 0[Y]	25.7 ± 16.4	1.19 ± 2.03
GA_3 at 25 ppm	Date 1	6.11 ± 7.23	43.6 ± 12.4	14.9 ± 15.3
	Date 2	3.41 ± 6.45	35.9 ± 13.5	30.3 ± 23.3
	Date 3	4.81 ± 4.05	42.8 ± 6.4	21.5 ± 30.1
GA_3 at 50 ppm	Date 1	6.49 ± 7.78	31.1 ± 13.8	32.6 ± 29.4
	Date 2	4.24 ± 8.09	41.8 ± 6.3	25.5 ± 8.78
	Date 3	6.51 ± 10.1	39.0 ± 9.5	35.2 ± 15.9
Significance level:	'date'	NS	NS	NS
	'concentration'	0.01	0.001	0.001
	'interaction'	NS	NS	NS

[Y]mean ± confidence interval

Table 3. Effect of the concentration and the timing of foliar application of GA_3 on yield (Kg/tree) and alternate bearing index (I) across three consecutive years.

Treatments		Yield according to years of study			I (%)	Cumulative yield (2001+2002+2003)
		2001	2002	2003		
Untreated control		71.4±25.3[z]	12.3±10.0	13.5±4.4	59.2±12.0	97.2±31.1 (-)
GA_3 at 25 ppm	Date 1	79.5±10.7	9.9 ± 5.1	70.5±12.8	73.5±9.5	159.9±26.4 (94.6)[y]
	Date 2	74.2±7.7	14.1±8.4	18.0±6.3	60.5±14.8	106.3±20.1 (9.3)
	Date 3	79.1±13.9	14.8±5.0	97.0±5.4	62.5±13.6	190.9±30.8 (96.9)
GA_3 at 50 ppm	Date 1	69.9±16.9	9.7±2.9	29.6±15.1	66.7±15.0	109.2±30.2 (12.4)
	Date 2	75.8±14.0	15.9±8.1	29.3±9.0	59.0±10.2	121.0±32.7 (24.7)
	Date 3	71.1±18.8	14.4±6.4	33.4±5.1	54.3±13.3	118.9±20.6 (22.6)
Significance level:	'date'	NS	NS	0.001	NS	NS
	'concentration'	NS	NS	0.001	NS	0.01
	'interaction'	NS	NS	0.001	NS	NS

[z]mean ± confidence interval ; [y]percent increase relative to untreated control

Furthermore, overall, cumulative yield over the three years was greater in tree receiving GA_3 at 25 ppm with ≈ 152 Kg/tree compared to 117 Kg/tree for the 50 ppm GA_3 and 97 Kg/tree for untreated trees (Table 3) indicating that the 50 ppm concentration might be too high and may cause a too much of an inhibitory effect on flowering (Tables 1 and 4). However, the alternate-bearing index was not affected by these treatments.

Effect of the combination of GA_3 applied in the 'ON year' and urea applied in the 'OFF year' on yield, alternate bearing index, cumulative yield, number of fruit per tree and individual fruit weight

The treatment strategy using urea as a foliar application during the 'OFF year' to promote flowering and GA_3 in 'ON year' to suppress flowering had a great effect on yield, and alternate-bearing index. In fact, in 2002 which is the 'OFF year', yield of urea-treated trees (Table 5) was greater than that of controls or that of GA_3-treated trees (Table 3). Treatment strategy that gave the greatest yield is: GA_3 at 25 ppm at date 2 in 2001 and GA_3 at 50 ppm at Date 1 in 2001 combined with urea at 1% in 2002 with: 54.9 and 49.8 kg/tree respectively.

In 2003, yield was increased by GA_3 and particularly at 25 ppm GA_3 application at Date 2 and Date 3 but fruit number was greater in 25 ppm GA_3-treated trees regardless of the timing of application (Table 5). The increase in yield in 2003 was due to the combination of increased fruit number and that of increased fruit size (Table 6).

Furthermore, the alternate-bearing index was lower in treated trees than in untreated ones (Table 5).

In addition, average cumulative yield over the 3 years of experimentation was greatest in trees receiving 25 ppm (140 Kg/tree) followed by those receiving the 50 ppm (121 Kg/tree). Untreated trees had at least 27% less fruit (Table 5).

The improvement of yield during the 'OFF year' using urea at 1% can be explained by the increase in the number of flowers generated as a resulty of treatment. In fact, Ali and Lovatt (1995), Lovatt et al. (1988) and El-Otmani et al. (1998) reported that the application of urea during the flower initiation/differentiation stage increased yield as a result of an increase in fruit number which was in turn the result of increased inflorescence number and fruit set.

When yield was increased in 2002 as a result of urea treatment its increase in 2003 was lower than that observed for trees that received GA_3 all years (compare results on Tables 3 and 5) indicating a mitigation effect of urea supply on alternate-bearing.

Using GA_3 in 'ON year' followed by an application of urea in the following 'OFF year' during flower initiation/differentiation stage could be a possible solution to reduce the alternate bearing pattern of the 'Nour' clementine. However, effect on cumulative yield is not clear particularly since the number of years in the study is add and includes two 'ON years' and only one 'OFF year'.

In addition, work is needed to investigate possible nutritional imbalance as a causal effect of alternate bearing in the 'Nour' Clementine and also to confirm the results obtained using GA_3 in order to recommend a treatment strategy for this cultivar.

Table 4. Effect of the concentration and the timing of foliar application of GA_3 on fruit number per tree and individual weight (g/fruit) during 2001 and 2003

Treatments		Fruit Number / tree 2001	Fruit Number / tree 2003	Weight (g / fruit) 2001	Weight (g / fruit) 2003
Untreated control		855.5±276.9[z]	161±52.9	83.1±6.25	83.9±2.33
GA_3 at 25 ppm	Date 1	900.0±184.9	748±157.8	89.7±11.0	94.2±4.0
	Date 2	895.7±208.3	197±66.2	84.9±14.6	91.4±5.6
	Date 3	935.1±246.8	1073±71.9	86.1±11.2	90.4±6.4
GA_3 at 50 ppm	Date 1	775.4±226.2	336±170.8	91.3±9.54	88.0±4.6
	Date 2	927.3±290.9	359±113.1	84.7±15.6	81.6±1.1
	Date 3	831.9±146.7	388±56.6	85.1±12.3	86.1±2.6
Significance level:	'date'	NS	0.001	NS	0.05
	'concentration'	NS	0.001	NS	0.001
	'interaction'	NS	0.001	NS	NS

[z]mean ± confidence interval

Table 5. Effect of foliar application of urea (1%) in 2002 and GA_3 in 2001 and 2003 on yield (Kg/tree) and alternate bearing index (I) across three consecutive years

Treatments		Year of study 2001	Year of study 2002	Year of study 2003	Cumulative yield per tree (Kg)	I (%)
Untreated control		71.4±25.3[z]	14.7±3.78	3.9±2.23	90.0±16.1	66.3±19.4
GA_3 at 25 ppm or urea at 1% w/v	Date 1	79.5±10.4	16.1±3.4	19.1±11.5	114.5±13.5 (27.2)[y]	53.9±13.8
	Date 2	74.2±7.7	54.9±11.6	38.7±19.2	173.8±22.7 (90.0)	52.7±18.4
	Date 3	79.1±13.9	18.2±5.8	37.9±10.6	129.8±14.3 (44.6)	62.2±15.5
GA_3 at 50 ppm or urea at 1% w/v	Date 1	69.9±16.9	49.8±9.5	15.6±12.2	132.9±13.9 (47.8)	60.0±16.6
	Date 2	75.8±14.0	30.9±8.8	15.0±8.6	115.6±21.8 (28.4)	34.0±13.9
	Date 3	71.1±18.8	18.8±4.4	25.4±10.7	115.4±13.0 (28.2)	40.2±11.9
Significance level:	'date'	NS	0.001	0.05	0.05	NS
	'concentration'	NS	0.001	0.001	0.001	0.001
	'interaction'	NS	0.001	0.05	0.001	NS

[z]mean ± confidence interval [y]percent increase relative to untreated control

Table 6. Effect of foliar application of urea (1%) in 2002 and GA_3 in 2001 and 2003 on fruit number per tree and individual fruit weight in 2003

Treatment		Fruit number per tree in 2003	Weight (g/fruit) in 2003
Untreated control		44.5±25.6[y]	87.5±6.5
GA_3 at 25 ppm	Date 1	203.5±132.3	95.8±8.8
	Date 2	395.4±209.1	95.1±5.6
	Date 3	426.8±101.3	88.0±3.8
GA_3 at 50 ppm	Date 1	173.9±139.2	90.8±5.7
	Date 2	178.0±99.0	83.7±6.2
	Date 3	263.3±123.3	93.8±7.0
Significance level: ‘date’		0.05	NS
	‘concentration’	0.001	0.05
	‘interaction’	NS	0.05

[y]mean ± confidence interval

Acknowledgement

This work is part of ‘Memoire’ made by L. Benhamou at Institut Agronomqiue et Vétérinaire Hassan II, Complexe Horticole d’Agadir. The authors acknowledge the financial assistance from USDA and from GPA, Delassus and Agrisouss citrus export groups.

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